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CULTURING AND ECOLOGY OF DIAPTOMUS CLAVIPES AND CYCLOPS VERNALIS

Andrew Robertson, et al

Oklahoma University

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The results of a study dealing with effects of temperature on certain reproductive attributes of this species are presented. Temperature is shown to affect the longevity of the adult females as well as the size, carrying time, and probably total lifetime production of clutches. The results of this study indicate that certain of the reproductive attributes of the females are affected by the temperature of early life as well as the acclimation temperature.

The report includes the results from a study on the dynamics of a field population of D. clavipes. The durations of the various life history stages were estimated both from laboratory and field data. Life tables were constructed for the spring generation of this population as well as all generations in a reproductive year combined. The stages of greatest relative mortality were identified. The report also presents recommendations for culturing the cyclopoid copepod, Cyclops vernalis, and the results of studies concerning effects of temperature on certain reproductive attributes of this species.

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CULTURING AND ECOLOGY OF DIAPTOMUS CLAVIPES AND CYCLOPS VERNALIS

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ABSTRACT

This report presents the results of studies undertaken to develop a method of maintaining healthy, self-propagating, laboratory cultures of the freshwater calanoid copepod, Diaptomus clavipes. Recommendations are given as to the conditions of container size, type of culture medium, light conditions, temperature conditions, food type and quantity, frequency of replacement medium, and amount of disturbance suggested for culturing.

The results of a study dealing with effects of temperature on certain reproductive attributes of this species are presented. Temperature is shown to affect the longevity of the adult females as well as the size, carrying time, and probably total lifetime production of clutches. The results of this study indicate that certain of the reproductive attributes of the females are affected by the temperature of early life as well as the acclimation temperature.

The report includes the results from a study on the dynamics of a field population of D. clavipes. The durations of the various life history stages were estimated both from laboratory and field data. Life tables were constructed for the spring generation of this population as well as all generations in a reproductive year combined. The stages of greatest relative mortality were identified.

The report also presents recommendations for culturing the cyclopoid copepod, Cyclops vernalis, and the results of studies concerning the effects of temperature on certain reproductive attributes of this species. Temperature is shown to affect longevity of the adult female, egg carrying duration, clutch size, and egg development rate.

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CHAPTER 1

INTRODUCTION

Calanoid copepods, especially those from the genus Diaptomus, play a very important role in the ecosystems of lakes and ponds. In most of the larger bodies of water and many of the smaller ones, the diaptomids are very abundant, often the most abundant, microcrustaceans. Their role in these ecosystems seems to be primarily that of a herbivore. They, in turn, are fed on by carnivorous zooplankters and by fish, especially larval and juvenile stages. Thus, they are of vital importance as intermediate links in the food chain of our lakes and ponds and can be of significance in determining types and amounts of fish. Their vital role not withstanding, however, our knowledge concerning the general biology, environmental requirements, and interrelations within the ecosystem of this group of organisms is woefully inadequate. We are in no position to state what effect past changes in water quality have had on these organisms nor to predict consequences of future environmental alterations.

One of the major reasons, if not the main one, for our inadequate knowledge of this group is our inability to maintain self-propagating cultures under controlled conditions. This has acted as a major obstacle to efforts to ascertain environmental requirements and to determine the consequences of changes in water quality for populations of these animals.

Thus, in order to increase our knowledge of this group, a research program has been carried out which had the following objectives:

1. To learn how to keep healthy, self-propagating cultures of a diaptomid in the laboratory.
2. To study in controlled experiments the effects of environmental factors, especially temperature, on diaptomids.

3. To follow and analyze a field population of a diaptomid with special attention to the relation between population dynamics and environmental conditions.

This project was supported by a grant (USDI Grant 18050 ELT) from the Federal Water Pollution Control Administration (now part of the Environmental Protection Agency) of the U.S. Department of Interior to the University of Oklahoma Research Institute. This is the final report for this grant.

The grant was awarded for one year, extended for six months, and then continued for a subsequent year plus a six month extension. During the continuation period, the grant work was expanded to include a certain amount of research on a cyclopoid copepod. This entailed adding two objectives to the study. These were:

4. To develop dependable, reproducible methods for culturing a cyclopoid.

5. To explore the effects of certain environmental factors, especially temperature, on the cyclopoid.

The research on cyclopoids is also covered in this final report.

In order to simplify and clarify the presentation, this report is divided into five sections. Each section reports the results that correspond to one of the five objectives outlined above.

CHAPTER 2

THE CULTURING OF A CALANOID

Until quite recently very little success had been obtained in culturing calanoid copepods in the laboratory. Within the last decade or so, however, methods for culturing have been developed for a number of marine and estuarine species (see for example Conover, 1960; Jacobs, 1961; Mullin, 1963; Zillioux and Wilson, 1966; Corkett, 1967; Lewis, 1967; Mullin and Brooks, 1967; Corkett and Urry, 1968; Heinle, 1969; and Katona and Moodie, 1969). As far as can be determined, no comparable studies reporting on methods for culturing freshwater calanoids have been published.

The objective of the research reported in this chapter was to develop a reproducible method for maintaining healthy, self-propagating cultures of a diaptomid in the laboratory. Favorable conditions for culturing have been determined by varying the factors thought most likely to affect the culturing success and determining the effects of these variations.

The diaptomid selected for study was the species, *Diaptomus (Aglaodiaptomus) clavipes* Schacht. The reasons for this choice were the following:

1. This species is relatively large for a diaptomid. The large size facilitated counting and handling.

2. This species is found in small bodies of water such as ponds, as well as in large reservoirs. It was assumed that a form that inhabits small bodies of water would be easier to culture in the rather restricted volumes available in experimental containers than would more strictly limnetic forms.

3. This species is rather important in the ecology of the lakes and ponds of central North America. It is one of the three or four most commonly found diaptomids in the Plains States and the Southwest.

4. This species is found in many of the farm ponds in the vicinity of Norman, Oklahoma, and thus was readily available to the investigators.

Methods and Materials

As specified for each experiment, the animals used in this work were obtained either from laboratory stock cultures or directly from the field. The laboratory stock cultures were initiated with animals obtained from a farm pond in Range 3 west, Township 9 north, Section 11, southeast quadrant, in Cleveland County, Oklahoma, near the city of Norman. Animals from this pond were separated from all other macroplankters and placed in large containers of conditioned water. The young produced during experiments were also usually added to these cultures. The animals removed from these stock cultures for experiments were thus a combination of individuals added from field collections and from laboratory experiments as well as individuals produced within the culture.

Periodically a few milliliters from a food culture were added to each stock culture. The cultures were generally maintained at a temperature range of 24 to 26° C and under moderate illumination, although other conditions were used early in the work.

Where animals directly from the field were used in experiments, these animals were obtained mainly from the above pond. Unfortunately, this pond dried up during early April, 1970, and did not refill until the middle of May, 1970. During the interim, field animals were obtained from a farm pond located in Range 1 west, Township 8 north, Section 13, southwest quadrant, in Cleveland County. For any given experiments using animals from the field, care was taken to insure that all the individuals were obtained from the same population.

Field collections of copepods were made with a fine (No. 25 mesh), 10-inch diameter plankton net. The net was thrown out into the body of water and pulled to shore several times; or, if conditions permitted, it was towed through the water by a person wearing chest waders. The zooplankton was concentrated in an 8 dram vial attached to the smaller end of the net. All the organisms collected were transferred to a quart jar and transported to the laboratory where they were surveyed with a binocular dissecting microscope.

Unless otherwise specified, 350 ml glass containers (finger bowls with a 4 1/2-inch diameter) were used for the culturing experiments, while the immatures were reared in 100-ml glass beakers. In a few initial experiments, the culture water was changed at 7- to 10-day intervals, but in most experiments it was never totally changed. Evaporation losses were reduced by placing a cover over the mouth of each container, but it was still occasionally necessary to make up losses by adding distilled water.

Some consideration was given in this work to what type of water should be used for culturing. Three types of culture media (i.e., conditioned water, distilled water, and natural pond water) were tested. Conditioned water was prepared by placing tap water in 3.8-l glass containers and aging it for two weeks prior to use. The natural pond water was obtained from the pond in which the animals were collected and was filtered twice through a double layer of No. 25 silk bolting cloth before being placed in the culture containers. The distilled water was the product of a general-use laboratory still and was of rather low quality. Except where specified otherwise, conditioned tap water was used in this work.

During experiments unless otherwise specified, the animals in each container were examined daily. Any individuals found dead were isolated on a depression slide and examined microscopically to determine the sex. Each dead individual was replaced by an individual of the same sex. Each newly gravid female was isolated in a drop of water on a depression slide. A drop of methyl cellulose was added to immobilize her, and a dissecting microscope was used to count the number of eggs in her clutch. After the eggs had been counted, the females were either returned to their culture containers or were placed in separate 100-ml beakers, containing 70 to 80 ml of pond water, until their eggs hatched. After hatch, the females that had been placed in 100-ml beakers were returned to their original culture containers.

No attempt was made to isolate the immature animals on a daily basis during any experiment since the total number of young involved precluded such a procedure. Instead, when information on the young was gathered at all, a total count as well as an estimate of the developmental stage of each animal was made by observing the animals in well-lighted surroundings. The estimates of developmental stage were made on the basis of differences in total body size and appearance and not on microscopic examination of specific morphological features.

The animals were fed from mixed laboratory cultures which had been seeded initially with water and contained organisms from the pond located in Township 9 north. The cultures were maintained in two 2-gallon aquaria. A small number of ammonium nitrate granules were added at infrequent intervals. These aquaria were maintained at approximately 26° C and under moderate illumination. The liquid used for feeding was obtained by removing a volume from one of these aquaria and filtering it twice through a piece of No. 25 silk bolting cloth. Some consistency in the amount of material being added at each feeding during an experiment was maintained by using distilled water to adjust the concentrations of the feeding solutions to a constant light transmittance (85%) in a Hach Photometric Colorimeter. No qualitative determinations were made of the organisms present in the feeding liquid; but periodic, cursory examinations revealed that there were several types of algae and protozoans present. As the water in the experimental containers was usually not changed during the experiments, food organisms obviously must have reproduced in the containers. Thus, while approximately the same amount of food culture was added to each container during an experiment, different amounts of food may have been available in the different containers, especially later in an experiment, because the separate containers had experienced differences in the growth and reproduction of their endogenous food cultures.

The constant temperature rooms and chambers in which all of the experiments were conducted had automatic controls that permitted independent regulation of both light and temperature on a 24-hour basis. Light intensities were recorded with a Weston Light Meter, while all water temperatures were measured with a thermometer graded in degrees Celcius and correct within ± 1.0° C. Air temperatures in the constant temperature rooms and chambers were usually within ± 1.5° C of those temperatures set on the controls. The temperatures of the waters in the culture vessels were normally within ± 1.0° C of the desired temperature.

Selection of Culturing Conditions

This section is structured in a series of subsections, each of which deals with a different factor of importance in culturing. Such structuring was deemed desirable since the individual experiments often produced data that could be applied to the study of more than one of the culturing factors.

The experiments presented in the following subsections are much too limited to provide statistically defensible results concerning the effects of the culturing factors. However, it should be borne in mind that the objective of this research was to develop a method for culturing D. clavipes. Thus, the work undertaken was restricted to that judged necessary to develop such a method. In the next chapter a more rigorous consideration of the effects of temperature will be presented.

Container Characteristics

It was felt that the culture vessels should satisfy the following requirements: 1) the containers should not appreciably affect the composition of the culturing medium; 2) the containers should facilitate observations on and manipulation of the animals; and, most importantly, 3) the animals should exhibit reasonable survival and reproductive rates in the containers. To satisfy the first requirement glass containers were used. The second requirement was met by using only wide-mouth containers made of clear glass. With these restrictions set up, the only other question was what the volume of the cultures should be to ensure good survival and reproduction.

To study this question these properties were measured for animals maintained in five different volumes of culture media, i.e., 50, 250, 1000, 3000, and 20,000 ml. Containers were selected so that the depth of water was approximately the same in the different cultures. This meant that the ratio of volume to surface area was approximately constant for all cultures, and so the exchange with the atmosphere and the concentrations of the dissolved gases should not have varied greatly among the cultures.

The cultures were maintained in the laboratory at a temperature of 23 ± 3° C and with a light cycle of approximately 12-hours light and 12-hours dark. They were fed 4 ml from the mixed algal cultures at intervals of 2 to 3 days. In the containers of the three larger sizes, small quantities of distilled water were added as needed to keep the volumes stable. In the containers of the two smaller sizes, it was necessary to remove small quantities of the media occasionally to keep the volumes constant.

Three replicate cultures were maintained for each volume, so there were 15 cultures in total. Each culture was initiated with the addition of 5 adult males and 5 adult females of D. clavipes. Survival and reproduction were determined by periodically counting the number of adults, of total copepodites, and of egg clutches in each container.

In the 50- and 250-ml containers these counts were always made by direct examination. However, in the larger containers the numbers became too great for this approach to be practical. Thus, after the first four examinations, the counts for these larger cultures were estimated through the use of a subsampling technique. This consisted of obtaining a subsample by inserting a piece of clear plastic tubing, having an inside diameter of 3 cm, to the bottom of each culture container. The tube was then stoppered on top and sealed, by means of a plastic Petri dish, at the bottom. The subsample was withdrawn from the culture and the contained animals and clutches counted. After counting the entire subsample was returned to its container. Three, four, and five subsamples were taken from the 1000-, 3000-, and 20,000-ml containers respectively. The locations on the surface of the cultures where the tubing was inserted were chosen with the aid of a random numbers table so that the subsampling was carried out randomly. Tables 1, 2, and 3 present respectively the average numbers of copepodites, of adults, and of egg clutches on a series of dates for each culture size.

The 50-ml cultures were very unsatisfactory. No egg clutches were ever noted, and after two weeks no copepods were left in the containers at all.

For all the other culture sizes some reproduction was noted. However, the 250-ml containers were also unsatisfactory. A few clutches were noted on the first two dates the cultures were examined. After this, however, no eggs were ever found. The cultures increased in size somewhat when the nauplii from the early egg clutches grew to where they were counted as copepodites, but the last time the containers were examined all three cultures had gone to extinction. It was obvious that the conditions in these containers were not satisfactory for the development of self-propagating cultures.

The three larger culture sizes all seemed to be satisfactory for culturing. While they exhibited marked fluctuations in numbers of copepodites and of clutches, the population levels stayed at high enough levels to indicate that satisfactory culturing conditions were present.

The largest numbers of individuals were produced in the 20,000-ml cultures. However, the highest densities of individuals seemed to develop in the 1000-ml cultures. As the same amount of food was added to each culture, it was not surprising that the 1000-ml cultures had generally higher densities than found in the larger cultures. The fact that more individuals were produced in the larger containers than in the 1000-ml ones shows, however, that even with the same amount of food added the carrying capacities were higher in these containers.

Table 1. TOTAL COPEPODITE POPULATION SIZE (TOTAL NUMBER AND NUMBER PER LITER) ON A SERIES OF DATES FOR D. CLAVIPES CULTURES MAINTAINED IN CONTAINERS OF 5 DIFFERENT VOLUMES. (EACH VALUE IS THE AVERAGE OF THREE REPLICATES.)

Sampling Date (1970)	Container Volume (ml)									
	50		250		1,000		3,000		20,000	
	Total No.	No./l	Total No.	No./l	Total No.	No./l	Total No.	No./l	Total No.	No./l
24-VI	10.0	200.0	10.0	40.0	10.0	10.0	10.0	3.3	10.0	0.5
27-VI	8.3	166.0	8.7	34.8	10.0	10.0	9.0	3.0	7.7	0.4
1-VII	0.7	14.0	8.0	32.0	11.7	11.7	11.7	3.9	11.7	0.6
3-VII	0.3	6.0	6.3	25.2	14.3	14.3	9.7	3.2	11.7	0.6
7-VII	0.0	0.0	5.0	20.0	16.7	16.7	9.3	3.1	-	-
15-VII*			-	-	24.0	24.0	58.7	19.6	331.7	16.6
20-VII			8.7	34.8	38.0	38.0	43.7	14.6	387.3	19.4
27-VII			6.0	24.0	47.7	47.7	67.3	22.4	328.7	16.4
1-VIII			3.7	14.8	41.0	41.0	47.3	15.8	313.0	15.7
31-VIII			0.0	0.0	25.3	25.3	40.7	13.6	123.0	6.2

* Subsampling initialed in 1,000-, 3,000-, and 20,000-ml cultures

Table 2. ADULT POPULATION SIZE (TOTAL NUMBER AND NUMBER PER LITER) ON A SERIES OF DATES FOR D. CLAVIPES CULTURES MAINTAINED IN CONTAINERS OF 5 DIFFERENT VOLUMES. (EACH VOLUME IS THE AVERAGE OF THREE REPLICATES.)

Sampling Date (1970)	Container Volume (ml)									
	50		250		1,000		3,000		20,000	
	Total No.	No./l	Total No.	No./l	Total No.	No./l	Total No.	No./l	Total No.	No./l
24-VI	10.0	200.0	10.0	40.0	10.0	10.0	10.0	3.3	10.0	0.5
27-VI	8.3	166.0	8.7	34.8	10.0	10.0	9.0	3.0	7.7	0.4
1-VII	0.7	14.0	8.0	32.0	8.0	8.0	8.8	2.9	9.1	0.5
3-VII	0.3	6.0	6.3	25.2	11.6	11.6	8.6	2.9	9.2	0.5
7-VII	0.0	0.0	5.0	20.0	10.5	10.5	6.8	2.3	-	-
15-VII*			-	-	6.5	6.5	19.3	6.4	70.3	3.5
20-VII			8.7	34.8	30.0	30.0	28.1	9.4	189.0	9.5
27-VII			6.0	24.0	41.4	41.4	43.1	14.4	136.4	6.8
1-VIII			3.7	14.8	26.7	26.7	37.2	12.4	98.9	4.9
31-VIII			0.0	0.0	21.3	21.3	30.8	10.3	81.1	4.1

* Subsampling initiated in 1,000-, 3,000-, and 20,000-ml cultures

Table 3. THE NUMBER OF CLUTCHES (TOTAL NUMBER AND NUMBER PER ADULT) BEING CARRIED ON A SERIES OF DATES IN D. CLAVIPES CULTURES MAINTAINED IN CONTAINERS OF 5 DIFFERENT VOLUMES. (EACH VALUE IS THE AVERAGE OF THREE REPLICATES.)

Sampling Date (1970)	Container Volume (ml)									
	50		250		1,000		3,000		20,500	
	Total No.	No./adult	Total No.	No./adult	Total No.	No./adult	Total No.	No./adult	Total No.	No./adult
24-VI	-	-	-	-	-	-	-	-	-	-
27-VI	0.0	0.0	3	0.345	5	0.500	4	0.444	3	0.390
1-VII	0.0	0.0	1	0.125	4	0.500	5	0.568	4	0.440
3-VII	0.0	0.0	0	0.0	6	0.517	5	0.581	3	0.326
7-VII	0.0	0.0	0	0.0	2	0.190	5	0.735	11	-
15-VII*			-	-	-	-	-	-	-	-
20-VII			0	0.0	0	0.000	0	0.000	68	0.360
27-VII			-	-	4	0.097	15	0.348	27	0.198
1-VIII			-	-	9	0.337	3	0.081	33	0.334
31-VIII			0	0.0	4	0.188	13	0.422	0	0.000

* Subsampling initiated in 1,000-, 3000-, and 20,000-ml cultures.

It would seem that it can be concluded from this work that large containers are better for culturing than smaller ones and that, if the containers are too small, culturing will not be possible at all. The choice of container size will depend partly on the goals of the work being undertaken. It has been found that the large containers are too difficult to manipulate, clean, etc. to be used in experiments that require a lot of examination and care of the animals, and containers of the 1000-ml range are advised for such experimental studies. However, for general culturing to provide stock animals where less care is needed, the large containers are convenient, and 5- to 10-gallon aquaria have proven quite satisfactory for this type of use.

Culture Medium

The selection of a culture medium was made, in part, on the basis of a comparison of data collected by daily monitoring of the mortality and egg production that occurred among field-collected (designated generation F_0) animals maintained in the three different water described in the Methods and Materials section, i.e., distilled water, conditioned tap water, and pond water. An experiment comparing distilled and pond water was conducted using animals collected during November 1969. Separate series of six 350-ml containers were placed in each of three temperature rooms. Three of the containers in each room held distilled water, while the other three contained pond water. Three adults, 1 female and 2 males, were placed in each of the containers, and this number was maintained throughout the experiment by replacing all dead individuals. The containers were kept under moderate to low illumination. Photoperiod was adjusted to be approximately 12-hours light and 12 dark in two of the rooms (Rooms A and B). In the third room (Room C) the light was left on all the time as this condition was required for other work being conducted in that room. A water temperature of approximately 25° C was used in two of the rooms (Rooms A and C), while 21° C was used in the third (Room B). Mortality and reproduction among these animals were monitored for 40 days. Data for mortality and reproduction in conditioned water were obtained from a previous experiment that covered a 40-day period and used animals collected during the summer months of 1969. The latter experiment employed separate series of six 350-ml containers in each of 3 temperature rooms with illumination and water temperature conditions as described above.

Table 4 shows the mortality over the 40-day span of the experiments among the animals cultured in each of the three media. The animals cultured in the conditioned water medium had an average mortality rate of 72.9%, those cultured in distilled water 65.6%, and those cultured in natural pond water only 16.3%. Statistically, an arcsine transformation (Sokal and Rohlf, 1969) was used to run t-tests to test for equality between pairs of percentages. The results of those tests revealed that the average mortality rate in the pond water differed significantly ($p < 0.001$) from that of either of the other media, but the averages did not differ significantly between conditioned water and distilled. These results should be valid for making a comparison between distilled water and pond water, but the comparisons between either of these media and conditioned water are less reliable since the experiment with the latter medium was conducted at a different time and with animals caught at a different season than for the experiment using distilled and pond water.

The reproduction data collected during these experiments did not show any large differences for the results for the three media (Table 5). However, there were some indications of differences in mean clutch size. Surprisingly, the averages in distilled water were larger than those for pond water in all three rooms. The conditioned water averages were lower than those for either of the other media in all rooms. No explanation for the apparent superiority of distilled water for production of large clutches can be offered. It may be that a more complete study would not support this preliminary finding as the differences reported here are not statistically significant.

The averages for the number of days the clutches were carried in each medium do not show any substantial differences. However, they do show sizeable differences among the different rooms, with the time being shortest in Room C and longest in Room A. No definitive explanation can be provided for this observation. However, the rooms

Table 4. MORTALITY OCCURRING AMONG ADULT D. CLAVIPES CULTURED IN 3 TYPES OF CULTURE MEDIA OVER A PERIOD OF 40 DAYS.

Culture Media	No. Animals Used			Mortality (Numbers)			% Mortality		
	Females	Males	Total	Females	Males	Total	Females	Males	Average
Conditioned Water	58	75	133	40	57	97	69.0	76.0	72.9
Distilled Water	35	60	90	17	42	59	48.6	70.0	65.6
Pond Water	21	22	43	3	4	7	14.3	18.2	16.3

Table 5. REPRODUCTION OCCURRING OVER A SPAN OF 40 DAYS AMONG F_0 GENERATION *D. CLAVIPES* CULTURED IN CONDITIONED WATER, DISTILLED WATER AND NATURAL POND WATER MEDIA.

Room	Culture Medium	Total Number Clutches	Range of Clutch Size	Mean Clutch Size	Range of Time Clutch Carried (Days)	Mean Time Clutch Carried (Days)
A	Conditioned Water	18	6-28	13.50	1-11	3.61
A	Pond Water	18	7-27	15.83	1-8	3.39
A	Distilled Water	7	11-28	19.71	2-10	3.86
B	Conditioned Water	3	1-8	5.00	1-3	2.33
B	Pond Water	9	8-15	10.44	1-6	2.78
B	Distilled Water	7	8-21	14.00	2-4	2.71
C	Conditioned Water	4	4-12	7.75	1-2	1.25
C	Pond Water	10	7-19	10.50	1-3	1.70
C	Distilled Water	13	5-26	15.08	1-5	1.92

varied to a certain extent in temperature and to a considerable extent in light intensity and duration. Room C had continual light of moderately low intensity, and these conditions may have encouraged algal growth in this room. This could then have led to increased food supply, and thus shorter average times for the carrying of the clutches. The results obtained in this work suggested that the influence of light conditions should be explored further. This was done and the results are considered in the next subsection.

The results presented here indicate that, in general, pond water is to be preferred for culturing because a lower mortality rate was observed in this medium than in the other two. Although no controlled experiments were conducted, the superior qualities of pond water for culturing were also indicated by certain observations on development of the young in the different types of media. In an informal comparison of development in distilled and pond water, approximately 500 nauplii were kept in distilled water, while approximately 2000 nauplii were simultaneously maintained in pond water under the same conditions. None of the nauplii in the distilled water lived to maturity, while over one-quarter of those in pond water did. General impressions on development in conditioned water indicated only poor development, although some individuals did mature in this medium. The general conclusion arising from our consideration of the three types of media is that pond water should be used for culturing unless an alternate medium has been shown to be as good or better. Obviously, however, the pond water should be from a pond where the species to be cultured occurs, as chemical conditions vary greatly from pond to pond.

Light

The influence of light on culturing has been explored by measuring simultaneously under three light intensities certain reproductive and developmental attributes. The experiment was designed to measure the effects of temperature (as described in the next subsection) as well as light, and so four temperatures regimes were used. However, animals exposed to each light intensity were represented only once in each temperature room, so that the first order effects of temperature were balanced out under each light condition, and the results will be presented in this subsection as simply testing the influence of light. The average temperatures of the temperature rooms used were approximately 17.2° , 21.9° , 26.4° , and 31.0° C.

Six 350-ml containers, each containing 1 adult female and 2 adult males in pond water, were set up in each room. The animals used in these experiments were the offspring of laboratory-cultured animals.

To achieve the desired illumination conditions, two containers in each room were completely covered with aluminum foil providing conditions of complete darkness for the animals in these containers. Two other containers in each room were covered with aluminum foil except for the top, which was covered by a piece of wax-paper resulting in a light intensity at the surface of the water of 125 to 140 foot-candles. The final two containers were covered on the side and bottom with foil as with the other containers, but the top was covered by a 4 1/2 x 4 1/2 x 1/8-inch piece of plate glass. This provided light of 200 to 218 foot-candles at the surface of the water. The light conditions were adjusted to approximately 12-hours light and 12 dark.

The reproduction of the adults was followed for approximately 30 days and the development of the young until they died or matured. A summary of the results is presented in Table 6. In order to minimize the influence of individual animals on the results, a double-averaging procedure was followed in obtaining the data in this table. The results for a parameter were averaged for each container, and then these values were averaged separately for each light intensity resulting in an overall mean at each intensity for each parameter.

It is immediately apparent from the table that the results are based on the production of only a few clutches. During the experiment reproductive activity was at a low

Table 6. A COMPARISON OF THE AVERAGE VALUES (±SE) FOR CERTAIN REPRODUCTIVE AND DEVELOPMENTAL PARAMETERS FOR D. CLAVIPES INDIVIDUALS AT THREE DIFFERENT LIGHT INTENSITIES.

Light Intensity (foot-candles)	Total Number of Clutches	Average Number of Clutches Per Container	Average Clutch Size	Average Percent Hatch	Average Days Clutch Carried	Average Days For Development To Adult
200-210	10	1.3 (±0.5)	11.2 (±2.4)	82.9 (±4.5)	2.4 (±0.5)	34.3 (±3.3)
125-140	6	0.8 (±0.3)	11.5 (±0.9)	59.1 (±8.3)	2.6 (±0.4)	21.7 (±1.8)
0	8	1.0 (±0.2)	12.2 (±2.6)	43.0 (±14.1)	1.8 (±0.5)	27.0 (±1.8)

level, probably because the food was of rather poor quality at that time. (This problem will be discussed further in the subsequent section on food.) Thus, only large differences in the influence of light intensity could have been detected.

No very large differences in the effects of the different intensities are evident from the results. There is some indication that the percent hatch increases with intensity and also that development time may be lowest at the medium intensity. However, there are too few data to establish these trends statistically, and overall it seems prudent to state that there is little indication that light intensity has a major effect on culturing success. From this work it was concluded that the use of a moderate to low light intensity with a period of 12-hours light and 12-hours dark should be satisfactory for culturing.

Temperature

The effects of temperature on the reproduction of D. clavipes will be considered at some length in the next chapter. However, before such detailed work could be carried out, culturing techniques including the determination of a temperature for culturing had to be determined. Thus, a certain amount of preliminary experimentation on the effects of temperature was conducted and is reported in this subsection.

Two experiments were carried out to study the effects of temperature. One of these was an examination of the combined effects of light and temperature. This is the same experiment as that described in the preceding subsection on light intensity. However, rather than averaging the data with regard to light as was done there, in this subsection the data on developmental and reproductive attributes from that experiment have been averaged for each temperature (Table 7). As there were two containers at each of three light intensities in each temperature room, averaging the data this way balances out the first order effects of light.

Table 7. A COMPARISON OF THE AVERAGE VALUES (±SE) FOR CERTAIN REPRODUCTIVE AND DEVELOPMENTAL PARAMETERS FOR D. CLAVIPES INDIVIDUALS AT FOUR DIFFERENT TEMPERATURES.

Average Temperature	Number of Clutches	Average Number of Clutches Per Container	Average Clutch Size	Average Percent Hatch	Average Days Clutch Carried	Average Days Development To Adult
17.2	6	1.0 (±0.4)	14.1 (±2.5)	70.9 (±7.0)	3.8 (±0.3)	31.6 (±4.1)
21.9	4	0.7 (±0.2)	9.8 (±1.5)	75.1 (±12.4)	2.3 (±0.5)	23.6 (±2.0)
26.4	11	1.8 (±0.4)	10.5 (±1.1)	65.6 (±9.8)	1.5 (±0.2)	31.8 (±4.2)
31.3	3	0.5 (±0.2)	13.7 (±6.1)	11.1 (±11.1)	1.5 (±0.5)	23 (-)

The second experiment (Table 8) was carried out under the same conditions as the first with three exceptions: 1) light was not varied but was at moderate intensity for 12 hours a day, 2) animals captured in the field were used rather than laboratory-cultured animals, and 3) the temperatures were somewhat different.

As can be seen from Table 8, no reproduction at all was observed for animals maintained at approximately 10° C, although substantial numbers of clutches were produced at the other three temperatures used in this experiment. Comparing the clutch production at the three higher temperatures, there were many more clutches produced at 29.6° than at 26.2° or 22.0° C. The average clutch size and average percent hatch were also highest at this temperature, although the differences are not as substantial as with the numbers of clutches. Further, the average number of days the clutches were carried and the average number of days for development to an adult were lower at 29.6° C than at the other temperatures. Overall there seems substantial indication that 29.6° C aided reproduction and maybe development and thus that this temperature should be good for culturing.

A contrast to the foregoing results is evident in Table 7. The most obvious difference is that, even though the two experiments were of approximately equal duration, many fewer clutches were produced in the Table 7 experiment. The reason for this is not immediately obvious. However, the two experiments were conducted at different times, and it is suspected that differences in the quality and, to a lesser extent, quantity of the food cultures at the different times may have played an important role in causing the differences in reproductive rate.

In this latter experiment the lowest temperature is no longer 9.7°, but is 17.2° C. Reproduction took place at this temperature, although the clutch carrying times show indication of being longer at this temperature than at the higher ones. Also it will be noted that the highest temperature is almost two degrees higher in this experiment than in the other one. Clutch production and percent hatch show indications of being

Table 8. A COMPARISON OF THE AVERAGE VALUES (±SE) FOR CERTAIN REPRODUCTIVE AND DEVELOPMENTAL PARAMETERS FOR *D. CLAVIPES* INDIVIDUALS AT FOUR DIFFERENT TEMPERATURES.

Average Temperature	Number of Clutches	Average Number of Clutches Per Container	Average Clutch Size	Average Percent Hatch	Average Days Clutch Carried	Average Days Development To Adult
9.7	0	0.0 (-)	---	---	---	---
22.0	17	2.8 (±1.1)	11.1 (±0.8)	51.8 (±21.7)	3.7 (±0.8)	20.2 (±1.8)
26.2	20	3.3 (±1.1)	10.5 (±1.2)	68.3 (±13.6)	2.0 (±0.4)	25.7 (±6.9)
29.6	39	6.5 (±0.4)	16.5 (±2.0)	74.5 (±9.9)	1.8 (±0.1)	18.1 (±1.5)

reduced at this temperature, and it is likely that this is approaching the upper thermal limit for the species. (Preliminary results, not included in this report, for a study on the upper thermal limit for this species seem to confirm this.) In general, the temperature of 26.4° C appears to be the most favorable of those studied in this experiment.

From these studies it was concluded that a range of temperatures from about 30° to 20° C allows substantial reproduction and development. It may be that the optimum temperature is just under 30° C. However, it is recommended that a somewhat lower value, around 25° C, be used for culturing. Although a few degrees warmer might increase culturing rate to a limited extent, these temperatures approach the upper thermal limit, and it seems prudent to avoid this range. The detailed studies on temperature reported in the next chapter fortify this recommendation.

Food

In the work described so far the food consisted of small volumes taken from a mixed culture grown in the laboratory. As indicated in the previous subsections, questions arose concerning the use of this type of food. Questions also arose concerning the quantity of food that should be added. The present subsection presents the results from a series of experiments designed to learn more about the effects of food on culturing and to aid in the selection of the feeding conditions to be used for culturing.

An experiment was conducted to compare the suitability of the mixed culture food with that of several other types. Each of 25 beakers was filled with 1000 ml of filtered pond water and had 8 adult D. clavipes, four of each sex, added to it. Feeding took place every second day, at which time the containers each received 2 ml of a food suspension. While the volumes of the food additions were equal, the type of material added varied; for 10 of the containers 2 ml of the mixed culture was added, for 10 others 2 ml from a yeast culture was added, and for the remaining five 2 ml from a culture of Chlamydomonas sp. acted as the food. In an attempt to make the quantity of food material of these different types that was added somewhat equal, the suspensions were all diluted with distilled water to a common spectrophotometric transparency value of 70% at 420 millimicrons.

All but five of the beakers were exposed to a 12-hour-on 12-hour-off cycle of moderate illumination, and efforts were made to equalize the distance of the beakers from the fluorescent light source. Five of the beakers that received yeast as food were not exposed to any light--except for a very small time during the addition of food. These beakers were completely covered with aluminum foil and were added to the experiment 3 weeks after the other beakers were initiated. The experimental containers were all kept in a constant temperature room at approximately 21° C.

The total number of copepodites in each container was estimated on a weekly basis for the duration of this experiment. These estimates were based on counts from three subsamples which were obtained by using glass tubing as described previously. Also, after the first few weeks, separate estimates were made of the number of adults in each of these containers. The results from the counts are presented in Tables 9 and 10 respectively.

The immediate impression gathered from these tables is that the mixed culture furnished the poorest of the three types of food. All 10 containers to which this food was added during the experiment were devoid of copepodites by the end of the work. This result is attributed primarily to the quality, as a food for D. clavipes, of the material added from the mixed culture. The production of individuals in the copepod stock cultures while this experiment was in progress was very poor relative to the production at other periods. A microscopic examination of the mixed culture material showed very few living cells, and even the color of the material was noticeably paler than usual.

A contributing factor to the extinction of the populations in all the mixed culture containers was the relatively small volumes used. With food of poor quality, only a

Table 9. A COMPARISON ON A WEEKLY BASIS OF THE AVERAGE NUMBERS OF TOTAL COPEPODITES OF *D. CLAVIPES* (±SE) IN POPULATIONS FED FROM A MIXED FOOD CULTURE, FROM A YEAST CULTURE (ADDED TO BOTH LIGHT AND DARK CONTAINERS), AND FROM A CHLAMYDOMONAS CULTURE

Date	Mixed food	Yeast in light	Yeast in dark	*Chlamydomonas*
20/XI/70 (Start)	8.0	8.0	--	8.0
27/XI/70	7.8 (±1.3)	9.9 (±2.8)	--	7.6 (±3.2)
4/XII/70	7.5 (±1.9)	11.1 (±4.3)	--	7.0 (±3.6)
17/XII/70	14.0 (±2.3)	7.8 (±3.5)	8.0 (start)	9.4 (±4.0)
23/XII/70	11.6 (±1.6)	6.6 (±2.5)	17.4 (±2.2)	7.8 (±1.8)
29/XII/70	14.4 (±2.4)	10.8 (±2.2)	7.2 (±1.8)	6.2 (±2.6)
8/I/71	10.2 (±2.7)	9.6 (±1.3)	4.4 (±1.6)	8.2 (±1.9)
16/I/71	8.7 (±2.4)	7.8 (±1.8)	4.8 (±1.4)	9.8 (±2.4)
21/I/71	6.2 (±1.9)	6.8 (±2.2)	2.2 (±1.1)	5.8 (±1.6)
28/I/71	5.7 (±1.8)	6.6 (±1.1)	3.8 (±1.8)	6.2 (±1.7)
4/II/71	3.6 (±1.1)	5.6 (±1.7)	3.4 (±1.5)	5.8 (±1.6)

TABLE 9 (Continued)

Date	Mixed food	Yeast in light	Yeast in dark	Chlamydomonas
11/II/71	2.8 (±0.9)	6.0 (±2.0)	1.4 (±0.7)	6.2 (±1.6)
18/II/71	1.6 (±0.7)	3.6 (±1.0)	1.2 (±0.6)	5.6 (±1.5)
25/II/71	0.9 (±0.6)	4.2 (±1.1)	1.0 (±0.4)	6.6 (±1.3)
3/III/71	0.8 (±0.4)	6.6 (±2.5)	1.2 (±0.6)	8.0 (±1.6)
11/III/71	0.7 (±0.3)	4.8 (±1.7)	1.2 (±0.6)	7.2 (±1.6)
18/III/71	0.4 (±0.2)	5.6 (±1.8)	1.8 (±1.6)	6.8 (±2.0)
25/III/71	0.4 (±0.2)	4.6 (±1.8)	1.4 (±1.4)	10.6 (±5.4)
1/IV/71	0.2 (±0.1)	3.8 (±2.4)	0.6 (±0.6)	12.2 (±5.3)
8/IV/71	0.2 (±0.1)	2.4 (±1.3)	0.4 (±0.4)	11.2 (±5.0)
19/IV/71	0.0 (----)	3.8 (±2.2	0.6 (±0.6)	8.2 (±3.3)

Table 10. A COMPARISON ON A WEEKLY BASIS OF THE AVERAGE NUMBERS OF ADULT D. CLAVIPES (±SE) IN POPULATIONS FED FROM CULTURES OF A MIXED FOOD, OF YEAST (ADDED TO BOTH LIGHT AND DARK CONTAINERS), AND OF CHLAMYDOMONAS.

Date	Mixed food	Yeast in light	Yeast in dark	Chlamydomonas
17/XII/70	5.5 (±0.5)	3.8 (±0.9)	---	3.2 (±0.6)
23/XII/70	4.9 (±0.6)	4.2 (±0.6)	---	2.6 (±0.9)
29/XII/70	4.5 (±0.5)	2.8 (±0.9)	4.8 (±0.8)	2.2 (±1.0)
8/I/71	2.7 (±0.7)	3.6 (±0.9)	2.0 (±0.8)	2.4 (±0.2)
16/I/71	2.1 (±0.6)	1.8 (±0.9)	2.0 (±0.6)	2.0 (±0.6)
21/I/71	0.7 (±0.3)	2.8 (±1.1)	0.6 (±0.2)	1.8 (±0.9)
28/I/71	0.9 (±0.4)	4.0 (±1.5)	2.0 (±1.3)	2.2 (±0.7)
4/II/71	3.0 (±0.9)	4.6 (±1.6)	1.6 (±0.8)	5.2 (±1.4)
11/II/71	2.8 (±0.9)	6.0 (±2.0)	1.4 (±0.7)	6.2 (±1.6)
18/II/71	1.6 (±0.7)	3.6 (±1.0)	1.2 (±0.6)	5.4 (±1.4)
25/II/71	0.9 (±0.6)	4.2 (±1.1)	1.0 (±0.4)	5.4 (±1.5)

Table 10 (Continued)

Date	Mixed food	Yeast in light	Yeast in dark	Chlamydomonas
3/III/71	0.7 (±0.3)	4.6 (±1.3)	1.2 (±0.6)	6.0 (±1.3)
11/III/71	0.7 (±0.3)	3.8 (±1.1)	1.2 (±0.6)	5.8 (±1.1)
18/III/71	0.4 (±0.2)	4.6 (±1.2)	0.8 (±0.6)	5.6 (±1.0)
25/III/71	0.3 (±0.2)	3.6 (±1.0)	0.6 (±0.6)	5.6 (±0.9)
1/IV/71	0.2 (±0.1)	2.6 (±1.3)	0.2 (±0.2)	5.2 (±1.2)
8/IV/71	0.2 (±0.1)	2.4 (±1.3)	0.4 (±0.4)	4.4 (±0.7)
19/IV/71	0.0 (--)	3.0 (±1.5)	0.4 (±0.4)	5.2 (±1.7)

few individuals could be supported. What seemed to occur was that the populations fluctuated to a certain extent, and the carrying capacity of these containers was so low that these fluctuations often resulted in the populations being completely wiped out. Obviously, after extinction the population could not recover even though the food being added might actually be capable of supporting a few individuals on the average. Thus, although it was concluded in a previous subsection dealing with container characteristics that 1000-ml containers are satisfactory for culturing, this is really only true when food conditions are favorable.

The populations supported by additions of yeast to containers kept in the light or of Chlamydomonas did not all go to extinction during the experiment as did the mixed culture ones. Both materials seemed to serve as quite good food sources. Chlamydomonas may possibly have been somewhat superior as none of its populations had reached zero by the end of the experiment, while two of the cultures supported on yeast in the light had done so.

The relatively satisfactory culturing results with these two materials raised a question as to how much the success was due to the material added serving directly as food and how much the success was due to this material acting as a nutrient source for the growth of mixed cultures of food organisms in the culture beakers themselves. The beakers which received yeast as a food but were maintained in the dark were added to the experiment in an attempt to gather some information concerning this question. It was reasoned that with light excluded growth of food microorganisms within the cultures would be greatly reduced, and so any reduction in copepod population development in the dark containers compared with the results for the populations fed yeast and kept in the light should indicate the effects of the in situ growth. Such a comparison (Tables 9 and 10) shows large differences. The populations kept in the dark but fed the same food as the populations kept in the light did much poorer than the latter populations. By the end of the experiment only one of the five dark populations still had any copepodites. Thus, as expected, it appears that in situ growth plays a major part in providing food in the containers kept in the light.

The foregoing experiments in this subsection together with our general experience in other experiments pointed out several problems with the use for food of material taken from mixed laboratory cultures of algae and other microorganisms. The suitability of this material for culturing D. clavipes varied greatly with time. This was only to be expected as the types and numbers of the organisms in these cultures undoubtedly fluctuated greatly. Also, it was very difficult with this type of food to determine and control the exact amounts of material being added. The food culture medium was diluted to a constant light transparency at each food addition in an attempt to standardize the quantity of food added. However, as the shapes and sizes of the food particles as well as their quantity are known to affect light transparency, this procedure could only have been partially successful.

The problems with the use of the mixed culture material for food necessitated a search for a more suitable material. Yeast and Chlamydomonas were used in the preceding experiment partially to test their suitability. Both seemed adequate for some purposes, but they still posed problems of exactly controlling the amounts added and both required a fair amount of effort to maintain the fresh cultures required to provide good food material. Also, the favorable results obtained by culturing with yeast were found to be due, quite largely, to the endogenous cultures growing within the copepod culture beakers rather than to the yeast directly. This fact, which very possibly is also true for Chlamydomonas, further complicates the standardization of food availability for copepod populations maintained in separate containers in the light.

Toward the end of our work in developing methods for culturing, a food material which had been used successfully with Daphnia came to our attention. This material is basically a blend of commercially prepared fish food with dried grass. It was produced by blending 10 g of Purine trout food, 0.5 g dried alfalfa grass, and 250 ml of filtered pond water for 5 minutes at top speed in a Waring Blender. The blender was washed with an additional 50 ml of pond water, and the entire mixture strained through #20 bolting cloth screen. The material retained on the screen was discarded and the filtrate served as the feeding solution. This material was made up fresh every two weeks or so and kept in the refrigerator when not in use so that bacterial growth and other changes were retarded.

This food has the great advantage that it can be made up to the same specifications each time it is produced. This permits control of the relative amounts of food added to different cultures at one time or to one culture at various times.

Besides changing the type of food added, it was also decided at this point to change the frequency at which the water in the experimental containers was changed. Rather than maintaining the same medium throughout an experiment as had been done up to this point, the water was changed periodically during an experiment in the rest of our work. This procedure was initiated in order to cut down growth of food organisms within the cultures and thus to aid in minimizing the availability of food from sources other than the known quantities that had been added.

A preliminary study was conducted to determine whether the fish food solution would support D. clavipes populations at all and thus whether it deserved further investigation. Twenty cultures were initiated in 1-l containers by adding 4 pairs of adult copepods to each. These cultures were divided into 4 sets of 5 replicates each, and a different amount of food was added to each set. Five of the containers received 50 ml of the fish food solution per day, five received 10 ml per day, five received 1 ml per day, and five received no food at all. These cultures were maintained in a constant temperature room at approximately 21° C and under moderate light conditions of 12 hours on and 12 hours off. The water in each container was changed once a week.

The results from this experiment are presented in Table 11. It will be noted that in less than two weeks all animals in the containers receiving either 10 or 50 ml per day were dead. The water in these containers turned cloudy and gave off a bad smell. It seemed obvious that the animals died out because so much food material was added that the water became anoxic and putrid conditions developed.

Table 11. A COMPARISON OF THE NUMBERS OF *D. CLAVIPES* COPEPODITES PER LITER (±SE) ON A SERIES OF DATES IN CONTAINERS RECEIVING DIFFERENT AMOUNTS OF FOOD. (EACH VALUE IS THE MEAN OF FIVE REPLICATES.)

Date (1971)	Volume of food per day (ml)			
	0	1	10	50
12/IV (Start)	8.0	8.0	8.0	8.0
23/IV	2.0 (±0.7)	3.2 (±0.4)	0.0 (----)	0.0 (----)
29/IV	0.6 (±0.4)	4.0 (±1.5)	0.0 (----)	0.0 (----)
11/V	0.6 (±0.4)	3.6 (±1.7)	0.0 (----)	0.0 (----)

By the end of a month only three animals were left in the containers that received no food, and no egg production or other signs of reproduction had been observed in these containers. Thus, there was little evidence that the copepods could be supported by endogenous growth if food was not added.

The containers that received 1 ml of food per day initially showed a decline from the 8 animals added originally. However, females carrying eggs were often present in these containers and nauplii and young copepodites were commonly observed. These cultures showed every indication of developing self-propagating populations, and it was concluded that the use of the fish food solution as food showed promise. However, the results of this work pointed out that the amount of food added is of critical importance to culturing success and should be studied further.

To do this an experiment similar to the previous one but with lesser amounts of food added was carried out. Sets of replicate cultures were initiated in this experiment as in the previous one with the exception that only three sets of three replicates each were used. The environmental factors were also the same as in the previous work except for the feeding conditions. In the present experiment three containers received 0.1 ml, three 0.5 ml, and three 1 ml of the food solution once every other day.

The results from this experiment are presented in Table 12. The populations did quite well in all the containers fed 0.1 or 0.5 ml of the fish food mixture. For the cultures fed 1.0 ml, however, one container had lost all its copepods and another had only one copepodite by the end of the experiment. It seemed that even 1 ml of this food every other day was too much. Both of the other volumes of food addition seemed to be satisfactory.

A further study was carried out to explore the effects on D. clavipes of varying the volumes of fish food solution added as food. This work was conducted in quite a

Table 12. A COMPARISON OF THE NUMBERS OF D. CLAVIPES COPEPODITES PER LITER (±SE) ON A SERIES OF DATES IN CONTAINERS RECEIVING DIFFERENT AMOUNTS OF FOOD. (EACH VALUE IS THE MEAN OF THREE REPLICATES.)

Date (1971)	Volume of food every other day (ml)		
	0.1	0.5	1.0
8/V (Start)	8.0	8.0	8.0
14/V	11.0 (±0.6)	9.0 (±2.1)	6.7 (±2.9)
20/V	22.0 (±2.1)	26.3 (±12.0)	3.7 (±0.9)
27/V	10.3 (±2.4)	12.0 (±4.6)	2.7 (±1.5)
3/VI	6.3 (±0.9)	15.7 (±6.6)	4.3 (±3.0)
10/VI	6.0 (±1.2)	9.0 (±6.5)	2.7 (±2.2)

different manner than the three previous experiments, however. In this work the mortality and reproductive success were monitored for individual females.

To initiate the work a number of females that had been raised in stock cultures at 21° C were placed in separate 100-ml beakers each containing 80 ml of filtered pond water. Before being added to the beakers, these females had been examined for the condition of their ovaries. The appearance of the ovaries was judged to fall into one of three categories—clear, somewhat opaque, or very opaque. Very opaque ovaries signaled an animal that was almost ready for oviposition; after oviposition the ovaries were clear. Only animals with opaque or very opaque ovaries were used to initiate this study.

Two adult males were also added to each beaker, and these were replaced during the experiment if any died. The females were not replaced, however. The beakers were maintained in a 21° C constant temperature room under light conditions of moderate illumination and 12-hours light and 12-hours dark.

From the beakers set up in this manner, three groups of six were selected using a random number table. One group received 0.8 ml of fish food solution every third day, one group 0.4 ml every third day, and one group no food at all. This experiment was repeated using all three food conditions and then repeated a second time with the omission of the beakers to which no food was added.

Each experiment lasted 9 days and during this time each beaker was examined once a day. At examination it was determined for each container: 1) whether the female was still alive, 2) if so whether she was carrying an egg clutch, and 3) if so how many eggs were in the clutch. The results from these experiments are summarized in Table 13.

Table 13. A COMPARISON OF MORTALITY AND REPRODUCTIVE SUCCESS (±SE) FOR *D. CLAVIPES* FEMALES KEPT UNDER THREE DIFFERENT FOOD CONDITIONS. (EACH VALUE IS THE MEAN FOR THE RESULTS FROM SIX INDIVIDUALS.)

	Experiment	Food added every third day (ml)		
		No food	0.4	0.8
Number of animals dying during experiment	1	0	0	3
	2	0	0	3
	3	-	2	5
Number of clutches produced per animal per day	1	0.11 (±0.0)	0.41 (±0.02)	0.47 (±0.04)
	2	0.11 (±0.0)	0.39 (±0.02	0.39 (±0.03)
	3	- -	0.21 (±0.06)	0.23 (±0.06)
Mean number of eggs per clutch	1	18.3 (±1.3)	24.5 (±2.2)	26.9 (±3.4)
	2	19.0 (±2.5)	18.0 (±1.4)	24.8 (±1.9)
	3	- -	15.2 (±1.7)	16.5 (±1.9)

Mortality was substantially greater in the containers receiving 0.8 ml of food every third day than in the other containers. Probably this amount of food was still too great to avoid deterioration of the quality of the medium.

On the other hand, clutch production per animal per day was as great in the containers receiving 0.8 ml as in those receiving 0.4 ml but, as expected, was reduced in the containers to which no food was added. In these latter containers each animal produced one clutch and no more. Thus, if these initial clutches, which were presumably partially formed when the experiments were started, are excluded, the rate of clutch production under conditions of no food additions is zero. Mean numbers of eggs per clutch did not vary greatly among the different food conditions.

As an interesting sidelight of these experiments, it will be noted that mortality tended to be higher and both rate of clutch production and size of clutch tended to be lower in the third experiment. The explanation for these differences is not apparent, but their existence does interject a note of warning against automatically assuming replicatibility of experiments using even the supposedly standardized fish food solution.

As an adjunct to the second and third experiments of the preceding work, one extra set of containers was initiated and maintained during each experiment in exactly the same way as the regular sets of containers. The animals in these extra containers were fed 0.4 ml of fish food solution every third day. The only difference between the treatment of the individuals in these containers and those that received 0.4 ml in the regular experiments was that the fish food solution added to the extra containers had been made up 2 weeks before the start of the experiment instead of at the experiment's start as was the food added to the regular containers. The food for all containers was held in a refrigerator at all times when not in use.

The mortality and reproduction for the females in these extra containers are summarized in Table 14. The results for the comparable animals from the regular experiments are

Table 14. A COMPARISON OF MORTALITY AND REPRODUCTIVE SUCCESS (±SE) FOR D. CLAVIPES FED 0.4 ML OF EITHER FRESH OR OLD FISH FOOD SOLUTION. (EACH VALUE IS THE MEAN FOR SIX INDIVIDUALS.)

	Experiment	Type of food	
		Fresh	Old
Number of animals dying during experiment	2	0	0
	3	2	2
No. of clutches produced per animal per day	2	0.39 (±0.02)	0.42 (±0.02)
	3	0.21 (±0.06)	0.21 (±0.03)
Mean number of eggs per clutch	2	18.0 (±1.4)	20.1 (±1.4)
	3	15.2 (±1.7)	15.8 (±1.3)

included for comparative purposes. No substantial differences can be noted between the results obtained for the animals fed the two kinds of food. This work gives no indication that the older fish food solution was any different in its ability to serve as food than the fresh material. Thus, it seems reasonable to suggest that the practice of making up new food only every 2 weeks rather than more often probably has little influence on the results obtained.

Overall, it is concluded that the fish food solution provides an excellent food for culturing and experimentation with D. clavipes. However, the volume and frequency of food additions are of crucial importance. Too much food leads to high mortalities probably caused by deterioration of the quality of the medium. Food additions as small as a few tenths of a milliliter every third day seem quite adequate to support strong reproduction and growth. Changing the water every few days even in stock cultures is also recommended with this food to avoid build-up of nutrients and the deterioration of water quality.

Disturbance of Animals

No controlled experiments were conducted to study the effects of disturbance of the animals on culturing success. However, an attempt was made throughout the work to keep the disturbance as little as possible. Minimal disturbance of the immatures and adult males was accomplished by making observations without removing them from their containers. It was necessary to disturb the adult females on numerous occasions in order to isolate gravid individuals. Yet, the females appeared to be remarkably tolerant to both the frequent handling required to make microscopic counts of the eggs in their clutches and to the methyl cellulose used to immobilize them during the process. Individual females were frequently isolated two or three times, a few as many as eight times, for this purpose.

CULTURING SUCCESS

While the techniques for culturing that have been described in this chapter were being worked out, a study was initiated to determine if the use of the techniques as then available would lead to self-propagating cultures. The animals used to initiate the study were collected from a field population in November 1969. Adults from this collection were maintained in several 350-ml glass containers at a density of 1 female and 2 males per 250- to 300-ml volume of natural pond water. Each newly gravid female was transferred to an individual 100-ml glass beaker containing 70 ml of natural pond water. These females remained in these small beakers until their clutches hatched and then were transferred back into their previous 350-ml beakers. The immatures remained in and were allowed to mature in the 100-ml beakers. At maturity, some of the new adults were transferred into 350-ml beakers. The same density of animals and the same type and volume of medium as stated above were used to culture these adults. This procedure was repeated for each new generation. The animals were fed a couple of milliliters of the mixed culture food every 2 to 3 days. Water temperatures in the 24 to 26° C range in combination with moderate light intensities were used.

This study extended over an 8-month period (November 1969 to July 1970) and was terminated voluntarily. The cultures passed through six and were in the seventh filial generation at termination. Thus, it was concluded that the techniques employed were suitable for the development of self-propagating cultures. As our work has ascertained that the conditions for some of the factors can be improved over those used for this culturing, it is assumed that the employment of the full procedures developed in our work and summarized in the next section of this chapter will allow easy culturing and experimentation with D. clavipes. In fact, this has been borne out by further work on this species as reported in the next chapter of this report.

SUMMARY

A study has been conducted with the primary objective of developing a reproducible method of maintaining self-propagating cultures of the calanoid copepod _D. clavipes_ in the laboratory. Based on the results of this study the following culturing conditions are recommended:

1. _Containers_--Wide-mouth glass vessels over one-half full and containing at least one liter of culturing medium. For most purposes a volume of medium in the range of 2 to 5 liters is to be preferred.

2. _Culture medium_--Water from an environment that naturally contains _D. clavipes_. This water should be filtered several times through fine mesh netting to remove all multicellular animals and the larger algal cells.

3. _Light_--Moderate illumination (50 to 150 foot-candles at the water surface) on a cycle of 12 hours on and 12 hours off.

4. _Temperature_--Within a couple of degrees of 25° C.

5. _Food type_--An aqueous mixture of commercial fish food and dried alfalfa grass prepared by blending these materials as described in the report and then filtering the resulting material. Other food materials were found to be satisfactory for some purposes but the fish food solution is preferable in general because it is easy to produce with a reproducible composition.

6. _Food quantity_--Approximately 0.1 ml of the fish food solution once every 3 days. Both the volume and the frequency of feeding can be altered somewhat as long as the total amount of material added is kept low.

7. _Changing medium_--Once every week or two.

8. _Disturbance of animals_--Should be minimized.

Culturing success has been tested by determining how long several females and their progeny could be maintained in the laboratory. This test was carried out before all the above recommendations had been arrived at, however, and so the conditions chosen were not as suitable as those specified above. Even so, the animals were cultured through six complete generations and into the seventh when the test was voluntarily terminated.

CHAPTER 3

THE EFFECTS OF TEMPERATURE ON THE REPRODUCTION OF DIAPTOMUS CLAVIPES

Relatively few controlled laboratory studies on the effects of temperature on freshwater copepods have been reported, although Coker (1933, 1934a, 1934b, 1934c) carried out a series of significant experiments with cyclopoids in the early thirties. Recently, other workers, notably Comita (1965, 1968), and Siefken and Armitage (1968) with diaptomids and Smyly (1970) with a cyclopoid, have conducted laboratory studies that included some consideration of temperature effects on certain physiological properties.

The development of a method for culturing Diaptomus clavipes, as reported in the preceding chapter, opened up the possibility of studying the effects of temperature on the reproduction of this species. The present chapter presents the results from such a study. This work was carried out to increase our understanding and predictive capabilities concerning the consequences of temperature fluctuations on the population dynamics of this species in particular and diaptomids in general. Admittedly, there is no guarantee that laboratory data accurately reflect what actually happens in a natural population. For that reason, predictions based on laboratory data alone can only be used with great caution. However, the laboratory results can provide a quantitative basis upon which to design and intrepret field studies.

METHODS AND MATERIALS

Stock cultures of Diaptomus clavipes were established using animals obtained on May 19 and 21, 1971 from a farm pond near Norman, Cleveland County, Oklahoma.

The pond is located on the west side of Flood Street, 2.7 miles north of Robinson Street (Section 11, Township 10 N, Range 3 W). In the laboratory *D. clavipes* was separated from *D. siciloides*, which is also found in the pond, on the basis of the larger size and red antennules of *D. clavipes*. Between May 19 and 28, 1971, stock cultures were established at 14°, 21°, 27°, and 31°C in constant temperature chambers set for 12-hour alternating periods of light and dark. In the 14° and 21° chambers, water temperatures were quite stable, never fluctuating more than ±0.5°C. In the other two chambers, water temperatures routinely fluctuated ±1.0°C and occasionally ±2.0°C for short periods.

The pond from which the animals were originally obtained was highly turbid, so water for culturing was taken from a pond 3.5 miles east and 2.8 miles south of the intersection in Norman of Lindsey and Classen Streets (Section 13, Township 8 N, Range 2 W). Water was collected in 5-gallon plastic bottles as needed, approximately every 7 to 14 days. In the laboratory, the water was twice poured from one bottle to another, both times with a single thickness of No. 20 bolting cloth covering the mouth of each bottle. Beakers of filtered water were maintained on two occasions for periods of 14 and 16 days, respectively, and were "fed" on the same schedule as used for experimental cultures. The only contaminating microcrustacean was a small cyclopoid copepod which probably passed through the bolting cloth as eggs or early nauplii. In addition to protozoans and algae, several small rotifers and rhabdocoel flatworms were observed.

Beginning on June 14, 1971, immature copepodites from stock cultures were isolated as needed for experimental purposes in 250-ml beakers containing 150 to 175 ml of water. No effort was made to determine the stage of the copepodites, since no experimental observations were made until after the animals matured. The isolated copepodites were observed once daily until they reached sexual maturity. When an individual was found to have molted to an adult female, it was transferred to a 400-ml beaker containing 250 to 275 ml of filtered pond water and one or two adult males. The water level was maintained by additions as necessary, but since the beakers were covered with a cellophane kitchen wrap, addition of water was seldom necessary.

When copepodites were needed for isolation, a stock culture was filtered, resulting in the collection of many more copepodites than were necessary for isolation at any one temperature. Other workers (Marshall & Orr, 1952; Corkett, 1967; Mullin & Brooks, 1967; Katona & Moodie, 1969) have emphasized that successful culturing of planktonic marine copepods requires that disturbances of cultures be minimized. Therefore, when copepodites were isolated from one stock culture, some were transferred to a different temperature chamber where they matured and lived out their lives. This provided considerable acclimation time, especially in relation to that allowed by some others (e.g., Comita, 1965; 1968) for laboratory experiments on animals taken in plankton samples. These individuals, which hatched and developed through all nauplias and some early copepodite stages at a temperature other than that at which they were kept as late copepodites and adults, are identified in the discussions which follow.

The introduction of copepodites from a warmer temperature was necessary at 14° to have experimental animals maturing at that temperature. Despite the continuous presence of egg-bearing females in the stock cultures at 14° and the regular addition to these cultures of nauplii produced by experimental animals at 14°, very few copepodites were ever found in these cultures. Of the few found and isolated, only one female (A7) reached maturity. (One or two males also reached maturity.) Aycock (1942), working with Cyclops vernalis, reported both higher mortality at lower temperatures and development time inversely related to temperature. If both conditions are true for D. clavipes (the inverse relationship between development time and temperature was confirmed in this study), they would account, in part at least, for the absence of copepodites in the 14° stock cultures.

For this work, the cultures were fed known volumes of the blend of commercial trout food and alfalfa whose preparation was described in Chapter 2. This food was stored in a refrigerator to retard bacterial growth and was freshly prepared every 10 to 15 days as needed. Each experimental culture received 0.4 ml of this preparation every third day. In order to avoid great differences in the algal and bacterial cultures growing in the experimental beakers and to prevent an accumulation of detritus in these containers, the animals were transferred to clean beakers containing fresh water every ninth day.

In the preliminary stages of the study, the contents of the beakers were filtered through a small hand net when it was necessary to isolate the females for egg counting, to transfer the animals to a fresh beaker, or to reduce the water volume to count nauplii. It soon became apparent that this almost daily (and in some cases twice daily) filtering was not satisfactory for a number of reasons. In addition to causing frequent stress on the animals, individuals were occasionally lost or killed. Therefore, to minimize the chances of error introduced by handling, the females used in the experiments reported here were removed from the water only for egg counting and for transferring to a fresh beaker each ninth day. In both of these procedures, females were isolated by pouring them out of the cultures into a second beaker. Thereafter, water was poured off, leaving the female behind in progressively smaller volumes of water. In this way, trauma and possible physical damage were minimized. As described below, nauplii were removed from the cultures without disturbing the experimental animals.

From maturity to death, females were observed at least once daily and, as possible, two or more times each day. At least one, but often more, of the observations each day was made by placing the beaker under a microscope and determining the following: 1) whether or not the female was carrying eggs; 2) the condition of the female's ovaries (prior to oviposition, the ovaries become progressively more opaque, thus providing a warning that oviposition is imminent); and 3) the presence or absence of nauplii. At other times, observations were made with the naked eye simply by looking into the beaker and recording the presence or absence of a clutch, although it was often possible also to observe and record the condition of the ovaries.

When the observations with the microscope were made, the eggs in each new clutch were counted and the nauplii were removed from any beakers in which eggs had hatched. Nauplii were removed from the beakers with a pipette, leaving the adults behind, and were discarded or put into a stock culture after they had been counted. Water removed from the experimental beakers while removing nauplii was returned, minus nauplii, to the same beaker, thereby maintaining the water level without loss or dilution of the food.

To count eggs, females were isolated, as described above, in a small volume of water which was gently poured into a Petri dish. Because these animals swim constantly, it was necessary to arrest their movement. Simply pipetting all water from the Petri dish did not permit counting of eggs, since the females reacted with violent swimming motions when out of water. Therefore, after all water was removed, a drop of methyl cellulose (about 4% solution) was put on the female. The animal was almost totally immobilized and eggs could be counted with ease. Using insect pins, it was possible to rotate the female for both dorsal and ventral views of the clutch. After the eggs were counted, filtered pond water was added from a plastic rinse bottle to wash the methyl cellulose away. Females were rinsed two or three times, as necessary, to restore free movement and normal swimming. After the rinse water was pipetted from the Petri dish, the edge was dipped into the female's culture beaker, allowing the animal to swim away.

When a female put on eggs, or when a clutch hatched between two observations, the time midway between was used as an estimate of the time the event occurred. Often, an old clutch hatched and a new clutch was put on between two observations. In such a case, hatching was estimated as occurring at the one-third point and oviposition at the two-thirds point in time between the two observations; e.g., if a female had a clutch at 12:00 noon and at 6:00 p.m. was found to have a new clutch, hatching was estimated to have occurred at 2:00 p.m. and the oviposition at 4:00 p.m.

All statistical analyses and tests follow the procedures of Sokal & Rohlf (1969).

FEMALE LIFE SPANS AFTER SEXUAL MATURITY

Generally, it was possible to recognize dying females by several indicators, although some individuals, especially at the higher temperatures, died without exhibiting prior indications. The most frequently observed indications of approaching death were a noticeable slowing of the animal's feeding movements and less active swimming. In most cases at the two lower temperatures, the females grew progressively weaker, with the first indications begining as much as 10 days or more before death. These females swam slower and often settled to the bottom for variable lengths of time. On the other hand, lying motionless on the bottom was not invariably associated with old age or approaching death. At 14°, some females ultimately became so feeble that they were unable to rise from the bottom of the beaker except to respond spastically after being touched. Females sometimes remained alive in this condition for several days, their feeding movements reduced to an occasional fanning motion and the hemocoele pumping very slowly and irregularly. Undoubtedly, in a natural population, aging animals would be easy prey for predators; and it is unlikely any would survive long after the initial slowing processes began. Certainly, none would be expected to live for days after becoming too feeble to swim.

Other signs of the female's approaching death were detritus accumulating on the antennules or caudal setae and a growth, apparently of bacteria, over her body. It is not known whether the bacterial infection was the cause of death, or only occurred on animals already weak and dying, but in any event death invariably followed within days after this growth was first observed. Once begun, the bacterial growth quickly covered the female, giving a velvety appearance to those that lived more than a day or two. Other workers (Corkett & Urry, 1968) have made similar observations and found that addition of antibiotics to the cultures extended the longevity of the copepods. No antibiotics were used in the experiments reported here.

Table 15 presents the values observed for female life span after maturity at the different incubation and hatching temperatures as well as the mean for each temperature. The samples in the present study were too small to permit statistical analysis in terms of the female's hatching temperatures, but, by inspection of Table 15, it appears that adult life span is determined by the incubation temperature, with no obvious influences attributable to hatching temperature. Thus, analysis of variance was carried out using all data at each incubation temperature. Not unexpectedly, mean adult longevity was found to decrease significantly ($F=23.7$; $P<0.001$) with increasing temperature. Since the females apparently lived to physiological old age, the values reported represent estimates of the longest expected adult life spans and may not be an accurate indication of adult life spans in a natural population. The conclusion that physiological longevity is inversely related to incubation temperature, however, is no less valid.

CLUTCH SIZE AND TOTAL EGG PRODUCTION

The data for egg production at the four different temperatures are presented in Appendices A-1 through A-4. In three cases (second clutch of B20, Appendix A-2; second clutch of D3; and fifth clutch of D16, both Appendix A-4), the eggs in a clutch were not counted. In those three cases, the average value for that female is reported. In all other cases, the values reported are numbers of eggs actually counted. Accuracy of the counts probably declines slightly with increasing clutch size. The

Table 15. FEMALE LIFE SPANS AFTER SEXUAL MATURITY IN RELATION TO INCUBATION AND HATCHING TEMPERATURES

Incubation temperature (degrees C)	Mean (days) ±S.E.	Female's hatching temperature (degrees C)	Mean (days) ±S.E.	Individual	Days survival after sexual maturity
14	84.6 ± 7.84	14	103	A7	103
		21	82	A6	82
		27	---	---	---
		31	79.3 ± 11.57	A8	72
				A9	102
				A10	64
21	43.2 ± 5.57	14	---	---	---
		21	38.9 ± 6.86	B11	64
				B12	56
				B14	16
				B17	27
				B18	38
				B19	22
				B20	49
		27	51	B16	51
		31	54.5 ± 13.50	B13	41
				B15	68
27	27.0 ± 4.77	14	---	---	---
		21	17	C9	17
		27	30.3 ± 5.79	C7	51
				C8	45
				C13	21
				C14	16
				C15	26
				C16	23
		31	17	C11	17
31	19.2 ± 2.45	14	---	---	---
		21	25	D8	25
		27	16	D5	16
		31	21.5 ± 1.32	D3	16
				D11	13
				D16	31
				D17	11
				D19	17
				D20	25

eggs in larger clutches were very tightly packed, often with more than two layers of eggs, making large clutches difficult to count accurately. Clutches in which eggs were irregularly arranged within the clutch also were difficult to count accurately. When a clutch was accidentally dislodged from a female during counting, the clutch was broken up and the eggs counted individually as a check on accuracy. In almost all cases, the number counted in the intact clutch was correct. Occasionally, with larger clutches or those in which eggs were irregularly arranged the count made of the intact clutch was short by as many as two eggs. All errors discovered in this manner were low counts, so that the data reported, if biased, are slightly conservative.

With each of the appendices is an Anova table for comparison of mean clutch size among all the individuals incubated at the temperature considered in that appendix. An a priori orthogonal set of comparisons also was made, grouping individuals by hatching temperatures. Only at an incubation temperature of 21° (Appendix A-2) did mean clutch size not vary significantly among individuals with a common hatching temperature. At the remaining three incubation temperatures (Appendices A-1, -3, and -4) variation among individuals with a common hatching temperature tended to be as great or greater than that among groups of individuals with different hatching temperatures.

Table 2 summarizes the mean clutch sizes found at the various hatching and incubation temperatures. Figure 1, using the data from Table 16, reveals two interesting features of the mean clutch size of "native" females (those incubated at the same temperature as that at which they hatched) compared to "non-native" females (those incubated at a temperature other than their hatching temperature). At every incubation temperature except 31°, "native" females have the largest mean clutch size. Futhermore, for every hatching temperature except 31°, mean clutch size is maximized when the incubation temperature corresponds. Although the evidence is not conclusive, the female's hatching temperature apparently can affect mean

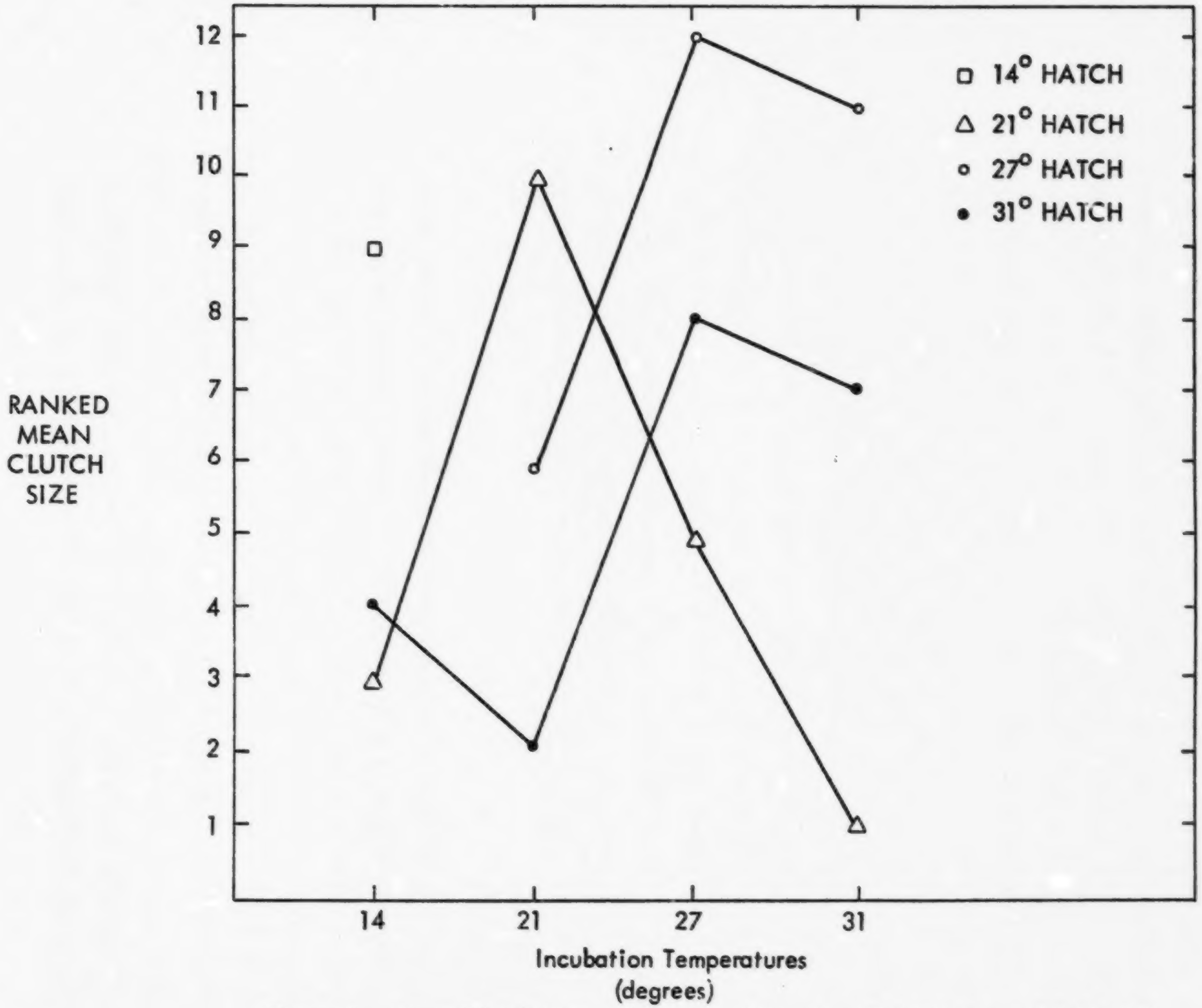

Figure 1. Mean Clutch Sizes of Groups of Females (Right Column of Table 16) are Ranked from Lowest to Highest (1 to 12) and Plotted Against the Temperature at which the Females Were Incubated.

Table 16. MEAN NUMBER OF EGGS PER CLUTCH IN RELATION TO INCUBATION AND HATCHING TEMPERATURES

Incubation temperature (degrees C)	Mean ± SE	Female's hatching temperature (degrees C)	Mean ± SE
14	21.5 ± 0.85	14	26.8 ± 1.14*
		21	17.6 ± 1.00*
		27	------
		31	20.2 ± 1.05
21	24.5 ± 0.77	14	------
		21	28.0 ± 0.80
		27	21.3 ± 1.76*
		31	15.9 ± 1.20
27	28.8 ± 1.07	14	------
		21	20.4 ± 1.00*
		27	30.0 ± 1.16
		31	25.9 ± 3.65*
31	21.1 ± 1.18	14	------
		21	13.6 ± 1.21*
		27	29.0 ± 1.91*
		31	21.3 ± 1.30

*Mean clutch size of a single female.

Anova table: Comparison of those females which were incubated at the same temperature as that at which they hatched.

Source of variation	df	MS	F	
Among incubation temperatures	3	568.5867	8.5327	P<0.001
Within incubation temperatures	195	66.6358		
Total	198			

Anova table: Comparison of all females at each incubation temperature.

Source of variation	df	MS	F	
Among incubation temperatures	3	780.8564	10.7858	P<0.001
Within incubation temperatures	291	72.3966		
Total	294			

clutch size, females producing larger clutches if they remain at the same constant temperature throughout life, and the largest clutches at each temperature being produced by these "native" females. Since the data are inconclusive, two analyses of variance have been carried out to examine the effect of temperature on clutch size (Table 16).

The first compares only those females "native" to each incubation temperature, all "non-native" females being omitted. For these "native" females, mean clutch size decreases in the order 27°, 21°, 14°, and 31° incubation. Differences in means are highly significant (F=8.5; P<0.001), but an a posteriori comparison of means by a Student-Newman-Keuls (SNK) test reveals that the means of females "native" to 14°, 21°, and 27° are not significantly different. Only the mean of females "native" to 31° differs significantly from the others.

The second analysis of variance in Table 16 includes data for all females at each incubation temperature, regardless of their hatching temperature. These overall means decrease in the same order as the means for "native" females and the differences are also highly significant (F=10.8; P<0.001). Applying a SNK test to these combined data, the means of 14°, 21°, and 31° are shown not to differ, only the mean for 27° being significantly higher.

Table 17 summarizes the data from Appendices A-1 through A-4 in terms of the mean total numbers of clutches produced during a lifetime at the various temperatures. Except at 21°, "non-native" females produced fewer clutches than did "native" females. However, the mean number of clutches was maximized at 21° incubation, regardless of the hatching temperature (Figure 2).

Analysis of variance (Table 17), comparing the mean total numbers of clutches produced at each incubation temperature, was first carried out using only data from females "native" to each incubation temperature (F=1.4;ns) and again using all

Table 17. LIFETIME TOTAL NUMBER OF CLUTCHES PER FEMALE IN RELATION TO HATCHING AND INCUBATION TEMPERATURES

Incubation temperature (degrees C)	Mean ± SE	Female's hatching temperature (degrees C)	Mean ± SE
14	8.2 ± 1.07	14	12*
		21	9*
		27	------
		31	6.7 ± 0.33
21	13.0 ± 2.36	14	------
		21	12.3 ± 3.35
		27	14*
		31	15.0 ± 3.00
27	10.2 ± 1.69	14	------
		21	7*
		27	11.3 ± 2.09
		31	7*
31	5.2 ± 0.88	14	------
		21	5*
		27	4*
		31	5.5 ± 1.18

*Lifetime total of a single female.

Anova table: Comparison of those females which were incubated at the same temperature as that at which they hatched.

Source of variation	df	MS	F	
Among incubation temperatures	3	57.5626	1.4295	ns
Within incubation temperatures	16	40.2663		
Total	19			

Anova table: Comparison of all females at each incubation temperature.

Source of variation	df	MS	F	
Among incubation temperatures	3	93.3140	3.4617	$P<0.05$
Within incubation temperatures	27	26.9555		
Total	30			

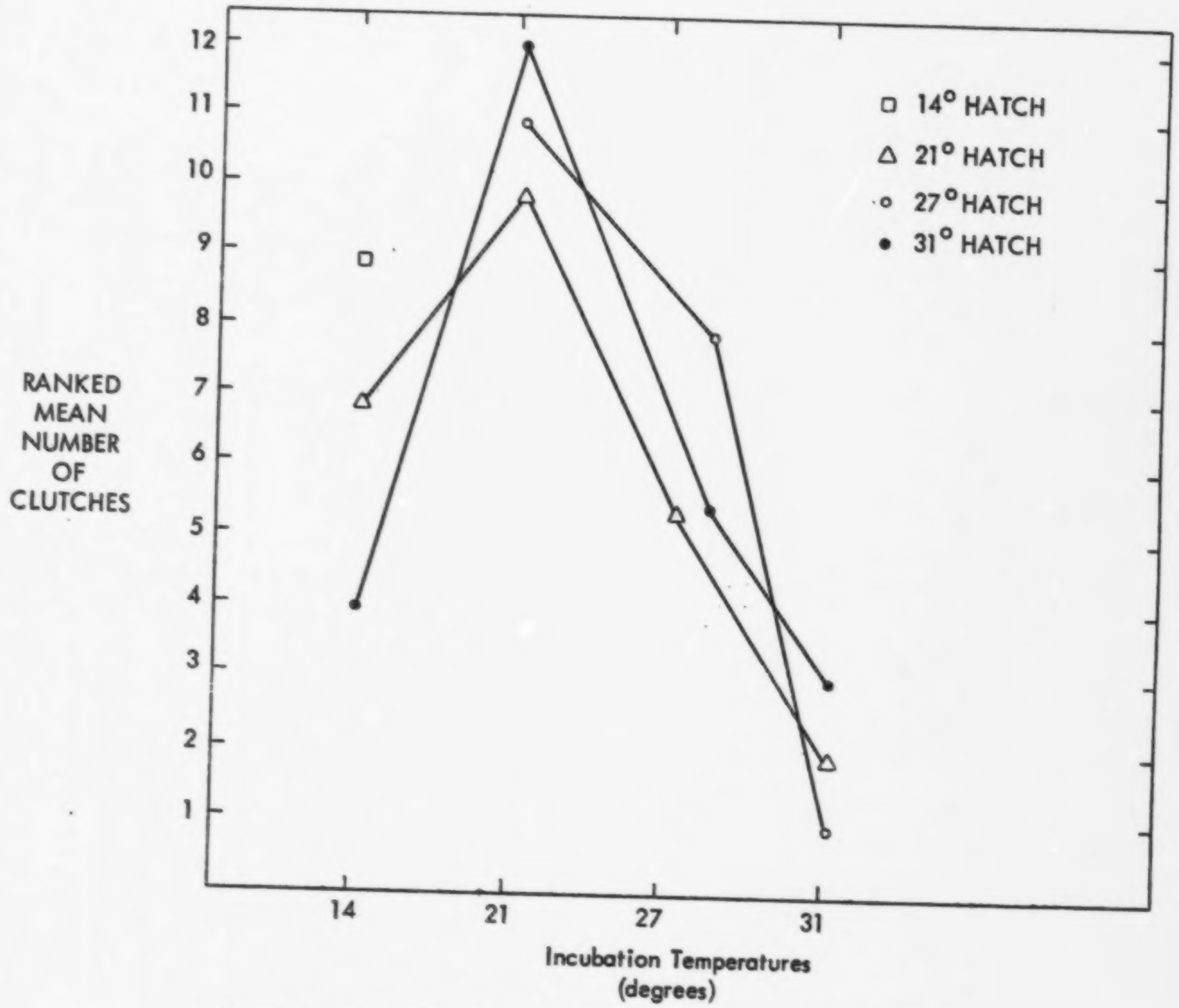

Figure 2. Mean Total Number of Clutches of Groups (Right Column of Table 17) are Ranked from Lowest to Highest (1 to 12) and Plotted Against the Temperature at which the Females Were Incubated.

data at each incubation temperature (F=3.5; P<0.05). In the latter case a SNK test shows no significant differences among means for 14°, 21°, and 27° incubation; or between the means for 14° and 31° incubation. The mean total number of clutches per female decreases in the order 21°, 14°, 27°, and 31° for "native" females. The order for the combined data at each incubation temperature is 21°, 27°, 14°, and 31°, which is almost the same order as that for mean clutch size, only 21° and 27° being reversed.

Combining the effects of clutch size and total production, we can now look at total lifetime egg production. At two incubation temperatures (21° and 31°), the highest mean lifetime total number of eggs is reported for a "non-native" female. As was true for the mean number of clutches produced, the mean total egg production is maximized at 21° incubation, regardless of hatching temperature (Figure 3 and Table 18). Analysis of variance is presented in Table 18, comparing only those females "native" to each incubation temperature (F=2.0; ns) and again using data for all females at each incubation temperature (F=3.7; P<0.025). As for the mean total number of clutches, the overall mean total number of eggs produced per female decreases in the order 21°, 27°, 14°, and 31°. The results of a SNK test are also the same, the data for 14°, 21°, and 27°; and for 14° and 31° being homogeneous sets.

The means of all first clutches, second clutches, etc. were computed for the data at each incubation temperature. To avoid excessive variability, these mean sequential clutch sizes were computed only so long as there were at least five females producing clutches. Analysis of variance was carried out for each incubation temperature (Appendices A-1 through A-4) and in no case was there significant variation. Combining all data regardless of incubation temperature (Table 19), no significant difference (F=0.73; ns) is found among the sequential clutches for the overall data. Thus, it is demonstrated that mean clutch size does not vary with the number of clutches the female has produced.

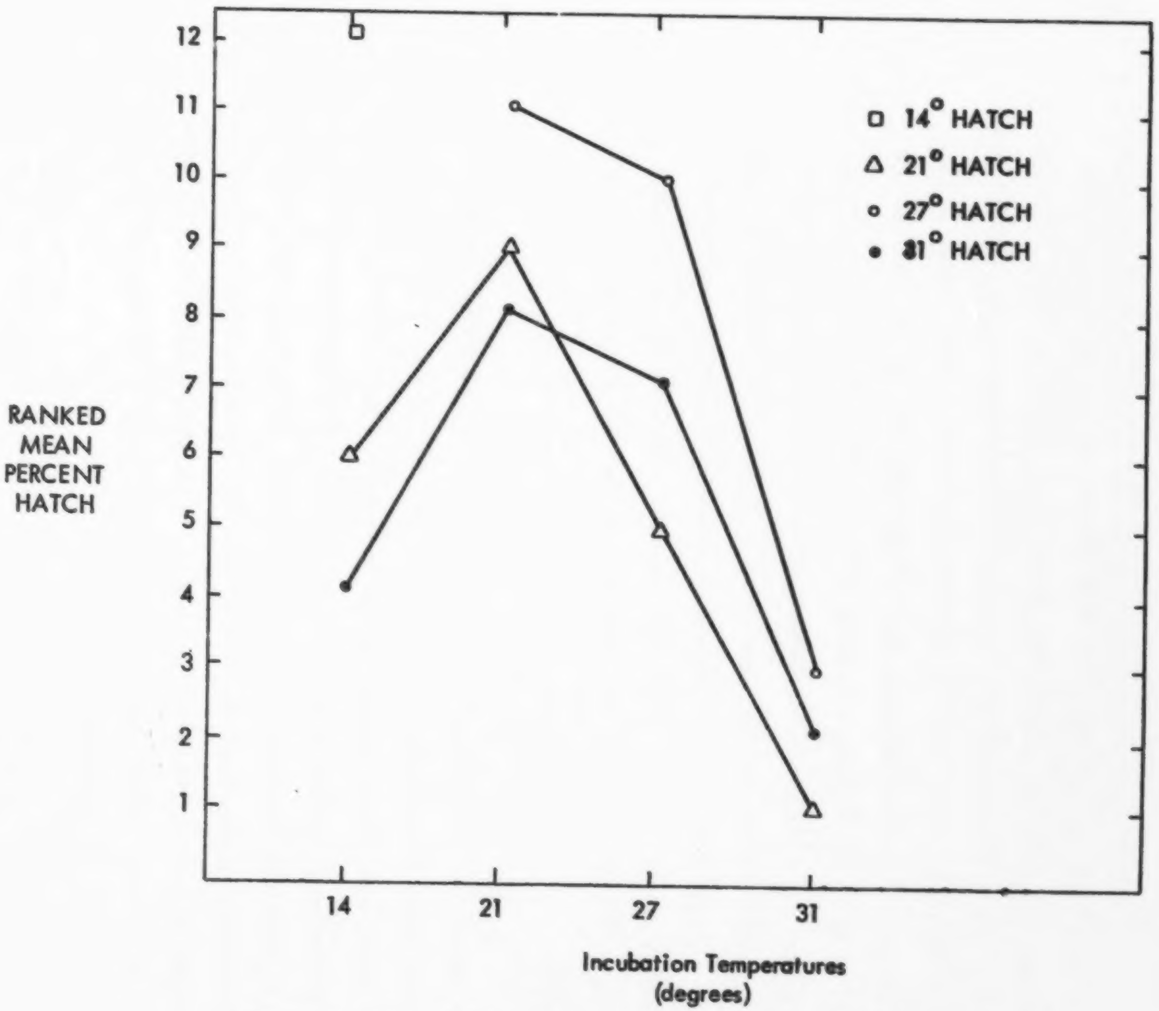

Figure 3. Mean Total Number of Eggs Produced by the Groups of Females (Right Column of Table 18) are Ranked from Lowest to Highest (1 to 12) and Plotted Against the Temperature at which the Females Were Incubated.

Table 18. LIFETIME TOTAL NUMBER OF EGGS PER FEMALE

Incubation temperature (degrees C)	Mean with 95% confidence limits	Female's hatching temperature (degrees C)	Mean with 95% confidence limits
14	164.0 98.69 - 272.4	14	322*
		21	158*
		27	----
		31	132.6 81.51 - 215.6
21	255.9 151.6 - 431.8	14	----
		21	255.8 112.2 - 582.8
		27	298*
		31	237.6 59.31 - 951.7
27	234.9 123.7 - 446.0	14	----
		21	143*
		27	266.4 107.9 - 657.9
		31	181*
31	94.03 53.43 - 165.5	14	----
		21	68*
		27	116*
		31	95.84 42.23 - 217.5

*Lifetime total of a single female.

An F_{max}-test indicates that the variances of the groups are heterogeneous. Therefore, a logarithmic transformation was made. The means and confidence limits above are the antilogarithms of the means and confidence limits of the transformed data. Using the transformed data, the following Analyses of Variance were carried out:

Anova table: Comparison of those females which were incubated at the same temperature as that at which they hatched--"native" females only.

Source of variation	df	MS	F	
Among incubation temperatures	3	0.2752	2.0294	ns
Within incubation temperatures	16	0.1356		
Total	19			

Table 18 (continued). LIFETIME TOTAL NUMBER OF EGGS PER FEMALE

Anova table: Comparison of all females at each incubation temperature.

Source of variation	df	MS	F	
Among incubation temperatures	3	0.3281	3.6741	$P < 0.025$
Within incubation temperatures	27	0.0893		
Total	30			

Table 19. COMPARISON OF MEAN SEQUENTIAL CLUTCH SIZES

Clutch no.	No.	Mean	Standard error
1	31	22.6	1.19
2	30	24.6	1.55
3	30	24.2	1.80
4	29	24.0	1.66
5	27	25.3	1.80
6	24	26.9	1.81
7	21	23.5	1.99
8	16	26.6	2.81
9	13	23.5	1.52
10	11	24.7	2.26
11	9	25.4	3.74
12	9	26.9	4.07
13	7	17.4	3.32
14	6	25.8	3.39
15	5	25.6	3.12
16	5	28.6	2.99
17	5	28.4	6.61

Anova table: Comparison of sequential clutches using all data, regardless of hatching or incubation temperature.

Source of variation	df	MS	F	
Among sequential clutches	16	62.3168	0.7330	ns
Within sequential clutches	261	85.0152		
Total	277			

RATE OF CLUTCH PRODUCTION

Although listed in chronological order, the data on the rate of clutch production presented in Appendices B-1 through B-12 are disjunct, data for some clutches being unobtainable for various reasons. For example, clutches were rather frequently knocked off prematurely, usually by the experimenter, but on occasion by the copepods themselves. In these instances no data on clutch carrying time or interval until the next clutch are reported for that clutch, although a value for the time from oviposition to oviposition is recorded. Early in the study, there were several instances in which a new clutch was not recognized as such until it was impossible to make a reasonable estimate of the time of oviposition, so that only the interval following such a clutch could be determined.

Since _D_. _clavipes_ females carry clutches until the eggs hatch, the cycle of egg production is naturally divided into two parts: the time the female is carrying developing eggs and the time between clutches during which there are no eggs. If these two parts of the cycle were to vary independently with temperature, it would be possible for the length of the overall cycles at different temperatures to be identical, despite having the two component parts quite different. Therefore, all three times (the length of time clutches were carried by the female, the length of the interval between successive clutches, and the length of the complete cycle from oviposition to oviposition) are reported here. Since the data are incomplete, the reported mean clutch carrying times plus mean intervals between clutches do not exactly equal the mean complete cycle times reported for each incubation temperature.

Three of the females incubated at 21° produced clutches of resting eggs. Since this was a completely different egg type and the resting eggs obviously were not carried until they hatched, the three time intervals when resting eggs were involved were compared to the three time intervals when clutches of subitaneous eggs were involved (Table 20). The mean time clutches of resting eggs were carried was significantly shorter (F=15.3; P<0.001) than the mean carrying time of subitaneous eggs. Since the mean intervals following clutches of the two egg types were not significantly different (F=0.18), the significant difference (F=8.5; P<0.005) between the two complete cycles can be attributed to the shorter carrying time of resting egg clutches. Since both the mean carrying times and mean lengths of a complete cycle were significantly shorter for clutches of resting eggs than for clutches of subitaneous eggs, data for these intervals were excluded from the 21° data for comparison with other incubation temperatures. For consistency, although the mean interval between clutches was not significantly different, intervals following resting clutches were also excluded from the 21° data used.

The data from each incubation temperature for all three time intervals were compared for variability among individuals (Anova tables are shown with Appendices B-1 through B-12). Significance (F=4.0; P<0.010) was indicated only for mean carrying times of individuals incubated at 27° (Appendix B-3). An orthogonal set of a priori comparisons was carried out for these data (Appendix B-3), with the result that the variability among individuals cannot be attributed to hatching temperatures as was variability in clutch size (see preceding section). Clutches apparently were produced at rates determined by the incubation temperatures alone. Therefore, all data from each incubation temperature were used for Tables 21,22, and 23, regardless of hatching temperatures.

When computing the mean clutch carrying times (Table 21) at the four incubation temperatures, data for clutches of resting eggs were excluded from the 21° data. However, sterile clutches could not be identified unequivocally and so could not be excluded. If the carrying time of sterile clutches differs, the effect of their inclusion in the data probably is slight, since the incidence of sterile clutches, as determined in the next section, appears to be low. A highly significant (F=261.0; P<0.001) inverse relationship between temperature and development time is apparent

Table 20. COMPARISON OF TIME INTERVALS OF RESTING AND SUBITANEOUS EGGS PRODUCED AT 21° INCUBATION

	Resting eggs	Subitaneous eggs	Analysis of variance results
Mean carrying time (hours)	39.0	48.2	F=15.3; $P<0.001$
Mean interval following clutches (hours)	9.7	10.6	F=0.18; ns
Mean complete cycle which included clutches (hours)	53.2	63.7	F=8.5; $P<0.005$

in Table 21. The mean for 31° is only slightly lower than the mean for 27°, and an SNK test indicates the two are not significantly different.

The relationship between temperature and the interval between successive clutches (Table 22) is not so straightforward. The longest interval is at 14° as was the longest development time. However, for the remaining three incubation temperatures, the relationship is direct. Differences in the means are highly significant ($F=15.7$; $P<0.001$), but a SNK test indicates that neither the means for 21° and 27° nor the means for 14° and 31° are significantly different. Therefore, the data indicate a fairly narrow range of intermediate temperatures in which the mean interval between clutches is minimized, with the interval increasing markedly at both higher and lower temperatures.

Similarly, the length of the complete cycle from oviposition to oviposition (Table 23) is minimal at the intermediate temperatures and higher at the extremes. The minimum is shifted toward the higher temperatures by the strong inverse relationship between development time and temperature, but the interval between clutches is sufficiently long at the upper extreme to make the complete cycle longer at 31° than at 27° despite the slightly shorter mean development time at 31°. Analysis of variance indicates highly significant ($F=113.3$; $P<0.001$) differences among the means of the four incubation temperatures. Comparison of these means by a SNK test indicates that the mean for each incubation temperature differs significantly from the means for all other incubation temperatures.

HATCHING SUCCESS

Data on hatching success are not presented for every clutch produced by the experimental females. Some of the clutches consisted of resting eggs, a few of which hatched up to two and one-half months after oviposition. No data were obtained on the hatching success of these clutches. Of those resting eggs that did not hatch

Table 21. COMPARISON OF MEAN CLUTCH CARRYING TIME AT FOUR INCUBATION TEMPERATURES

Incubation temperature (degrees C)	Mean (hours)	95% confidence limits
14	113.1	108.2 - 118.2
21	48.2*	45.7 - 50.9*
27	32.7	30.6 - 34.9
31	29.8	28.0 - 31.6

*Carrying times of clutches of resting eggs excluded.

An F_{max}-Test indicates that variances of the data for the four incubation temperatures are heterogeneous. A logarithmic transformation was made and an Anova carried out with the transformed data.

Anova table: Carrying times by incubation temperature. Data for clutches of resting eggs excluded from 21° data.

Source of variation	df	MS	F	
Among incubation temperatures	3	2.6887	261.0388	$P<0.001$
Within incubation temperatures	220	0.0103		
Total	223			

Table 22. COMPARISON OF MEAN INTERVAL BETWEEN CLUTCHES AT FOUR INCUBATION TEMPERATURES

Incubation temperature (degrees C)	Mean (hours)	95% confidence limits
14	35.4	23.3 - 54.0
21	10.7*	8.7 - 13.1*
27	11.5	9.2 - 14.3
31	22.9	17.0 - 30.8

*Intervals following clutches of resting eggs excluded.

An F_{max}-Test indicates that the variances of the data for the four incubation temperatures are heterogeneous. A logarithmic transformation was made and an Anova carried out with the transformed data.

Anova table: Intervals by incubation temperature. Data for intervals following clutches of resting eggs excluded from 21° data.

Source of variation	df	MS	F	
Among incubation temperatures	3	2.4857	15.6826	$P<0.001$
Within incubation temperatures	199	0.1585		
Total	202			

Table 23. COMPARISON OF MEAN EGG PRODUCTION CYCLE FROM OVIPOSITION TO OVIPOSITION AT FOUR INCUBATION TEMPERATURES

Incubation temperature (degrees C)	Mean (hours)	95% confidence limits
14	166.7	147.5 - 188.3
21	63.7*	60.0 - 67.7*
27	47.3	36.9 - 60.7
31	55.2	48.4 - 63.0

*Cycles which included a clutch of resting eggs excluded.

An F_{max}-Test indicates that variances of the data for the four incubation temperatures are heterogeneous. A logarithmic transformation was made and an Anova carried out with the transformed data.

Anova table: Cycles by incubation temperature. Data for cycles which included a clutch of resting eggs are excluded from the 21° data.

Source of variation	df	MS	F	
Among incubation temperatures	3	2.1762	113.3437	$P<0.001$
Within incubation temperatures	217	0.0192		
Total	220			

during the course of this study, none decomposed, so it is possible that these might have hatched had the study continued longer.

Pipetting the individual nauplii from the culture was not a completely satisfactory method to count nauplii; and, as a result, the data for some clutches were so confused that no estimate of hatching was obtained. However, the pipetting was the only method that did not traumatize the females and thus did not invalidate the data on other factors being studied. Normally, the nauplii were slightly pigmented and were easily visible immediately upon hatching. Occasionally, nauplii were produced which totally lacked pigment and therefore were almost invisible until they reached the third or fourth naupliar stage. Additionally, at 27° and 31°C, the water became quite cloudy with suspended material and algal growth during the last three or four days before the regular water change. On occasion, the water was cloudy enough to make finding nauplii extremely difficult even if they were distinctly pigmented. As a result, not all nauplii could be found and pipetted out at one time, and it usually required two or more observation periods for all nauplii to be removed. In this time, some no doubt died. Others may have been missed several times and then removed and counted with nauplii from a later clutch. In the latter case, a difference in naupliar stage sometimes distinguished the older nauplii from the newly hatched ones, but it was not always possible to determine positively whether the older nauplii came from the previous clutch or from an even earlier one. In a few cases, overlapping between two clutches was so great that no data are reported for either.

The hatching successes of individual females were tested by analysis of variance for differences among the individuals at each incubation temperature (Appendices C-1 through C-4). Differences among individuals were highly significant at three incubation temperatures ($P<0.001$ at 14° and 21°, $P<0.010$ at 27°). Only at 31° incubation, where the hatching successes were uniformly low, were there no significant differences among individuals. As shown in Figure 4 (constructed using the

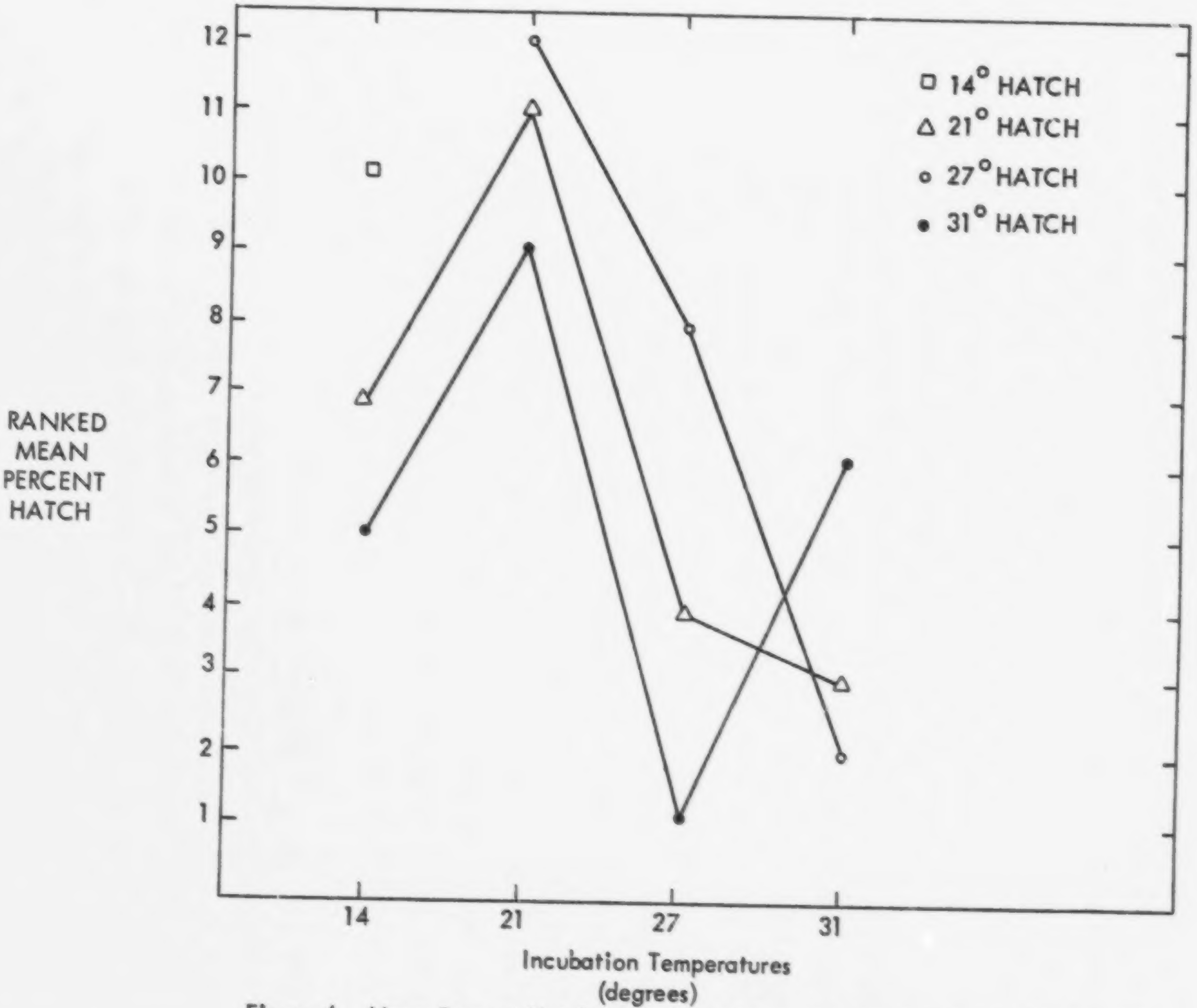

Figure 4. Mean Percent Hatch of Groups of Females (Right Column of Table 24) are Ranked from Lowest to Highest (1 to 12) and Plotted Against the Temperature at which the Females Were Incubated.

means presented in Table 24), the mean hatching success is highest for those females which hatched and lived their entire lifetimes at a single temperature except at 21° incubation. However, orthogonal sets of a priori comparisons (shown with the Anova tables in Appendices C-1,-2, and -3) were made for the 14°, 21°, and 27° data and indicate significant variability even among the females "native" to each of these incubation temperatures.

Whether all data at each incubation temperature are used or only the data for females "native" to each incubation temperature, mean hatching success is highest at 21° and is progressively lower at both higher and lower temperatures (Table 24). Considering only those females "native" to each incubation temperature and thereby eliminating any possible effects due to the individual's hatching temperature, analysis of variance indicates that mean hatching success varies significantly ($F=4.4$; $P<0.010$) with temperature (Table 24). An a posteriori SNK test, however, shows no significant differences among the means for 14°, 27°, and 31°, or between those for 14° and 21°. If all data, regardless of the female's hatching temperature, for each incubation temperature are used, significance is even greater ($F=9.0$; $P<0.001$), and the SNK test indicates that the mean for 21° is significantly higher than the means for the other three incubation temperatures, which do not significantly differ from one another.

Occasionally, a female lost her clutch before the eggs hatched. This happened especially during the counting of the eggs in a new clutch. Until they hatched, loose clutches were isolated in a small Petri dish containing water, and the resulting data are included here. For these loose clutches, it was usually possible to locate empty egg membranes after hatching, especially when they were all held together by the egg sac membrane, as was often the case. Since the water volume was small, about 20 ml, it was very easy to count all nauplii present. When the apparent hatching success was less than 100% and even when it apparently was zero, it was quite unusual to find either unhatched eggs or dead nauplii. In one case a nauplius

Table 24. HATCHING SUCCESS

Incubation temperature (degrees C)	Overall mean (%)	Female's hatching temperature (degrees C)	Mean (%)
14	66.8	14	82.3*
		21	68.0*
		27	----
		31	55.8
21	85.9	14	----
		21	85.8
		27	96.2*
		31	76.9
27	66.6	14	----
		21	51.7*
		27	71.7
		31	23.7*
31	53.3	14	----
		21	33.2*
		27	30.7*
		31	60.6

*Mean hatching success of a single female.

An Anova was carried out using the Arcsine transformation of the data for those females "native" to each incubation temperature. Data were not used for those females which were not incubated at the same temperature as that at which they hatched.

Source of variation	df	MS	F	
Among incubation temperatures	3	2065.1329	4.3675	$P < 0.010$
Within incubation temperatures	161	472.8348		
Total	164			

only half emerged from the egg was found dead. The impression, overall, was that in almost all cases all eggs hatch, but that some nauplii die very shortly thereafter and quickly decompose. In view of the usual delay between the time when the nauplii hatched and the time when they were removed from the culture and counted, the data reported here might more accurately be considered the percent hatching and surviving for approximately 12 hours thereafter.

DISCUSSION

The inverse relationship between adult longevity and temperature reported in this chapter is as expected for poikilotherms. The mean lifespans reported are quite similar to adult lifespans previously reported for other species cultured in the laboratory. Wilson & Parrish (1971) reported that females had a mean adult lifespan of 37.7 days when the marine calanoid copepod Acartia tonsa was incubated at 17.5°C. Smyly (1970) reported that females of the predaceous cyclopoid Acanthocyclops viridis incubated at 16-18°C had a mean adult longevity of from 30.8 to 57.3 days, depending upon the type of food.

Incubation temperature at or below 27° apparently has little effect on clutch size, at least among "native" females. This is in keeping with reports by other investigators. Considerable work has been done, particularly with various marine species, in an effort to discover what factors determine clutch size. The consensus is that clutch size is determined by the female's body size, not directly by temperature or food concentration (Corkett & McLaren, 1969; Smyly, 1968; Ravera & Tonolli, 1956), although it is conceded that under conditions of severe food shortage clutch sizes will decrease (Corkett & McLaren, 1969). Body size has normally been found inversely related to temperature (Coker, 1934a; Lock & McLaren, 1970; Smyly, 1970), but there have been reports both from the laboratory (Mullin & Brooks, 1970) and from the field (Deevey, 1964) of adult size being directly related to the food supply available to the immature stages. Obviously, the factors contributing to clutch size are multiple. Inheritance is probably also important and could cause the frequently significant variability reported here among females with common hatching and incubation temperatures (Appendices A-1, -2, -3, and -4).

As noted above, the literature generally indicates that clutch size is directly related to the body size of the female which in turn is partially determined by temperature (to which body size is inversely related). Accordingly, those females which hatched and underwent most of their development at a temperature lower than that at which incubated as late copepodites and adults would be expected to produce larger clutches than did females that lived exclusively at the higher temperature. Such is not the case with the data reported here, the data for females which hatched at 21° being particularly divergent from expectations (Figure 1). Unfortunately, samples are small and the females used were not measured. If the data are valid, reproductive potential is reduced unless the ambient temperature remains relatively constant throughout the life of the reproducing females. This, of course, is not the case in natural populations and such reduced reproductive potential in an environment of changing temperature appears to lack selective value. Therefore, these results should be verified by further experimentation before final conclusions are reached.

At the three lower incubation temperatures, the mean clutch size, the total number of clutches, and the total egg production seem to be unaffected by temperature, at least for females living their lives at a single constant temperature. This implies that, under these conditions, the reproductive potential of an individual is approximately the same, about 340 eggs, regardless of the temperature at which incubated. However, the potential for females in a natural population may differ considerably. Conover (1967) reported that female Calanus hyperboreus from laboratory molts were less fecund than were females taken from plankton samples. On the average, lifetime egg production by laboratory females was less than half that of females from the plankton. Similarly, Mullin & Brooks (1967) reported lower fecundity among laboratory females of two species of marine calanoids. In any event, for laboratory culturing, incubation temperature is important only as it affects survivorship to adulthood. On the other hand, while temperature over a certain range apparently does not greatly affect reproductive potential, it does affect the rate at which that potential is realized. If predation or other factors in a natural population operate to reduce the adult population before physiological old age, then temperature becomes an important factor as it affects the reproductive rate.

The question of whether females require more than one copulation to produce fertile eggs throughout their adult lifespans has been debated in the literature for many years (Ewers, 1936; Hill & Coker, 1930). Most marine calanoids apparently require only one copulation, although Wilson and Parrish (1971) have reported that *Acartia tonsa* will mate more than once in the laboratory. The intensity and duration of breeding activity among freshwater copepods have been estimated by counting the number of females carrying spermatophores (Comita, 1956; Comita & Anderson, 1959). The transfer of spermatophores was observed to last up to six months in some populations, which seems to imply repeated matings. *D. clavipes,* at least in the laboratory, is obliged to copulate each time a clutch is produced. Failure to copulate results in the extrusion of the egg material into a spherical sac without formation of individual eggs. This sac soon bursts and the egg material is dispersed. This would result in unacceptable waste of energy if it occurred with regularity in a natural population, but *D. clavipes* is largely restricted to small bodies of fresh water in which it is unlikely that any female would fail to encounter a suitable male.

Future studies of egg production by *D. clavipes* need not be concerned with the age of females with respect to clutch size, since this study has shown that clutch size does not change with the age of the female. This is not a safe general assumption for other copepod species, however. Smyly (1970) has reported not only that the number of eggs laid in successive broods by *Acanthocyclops viridis* diminishes with age, but also that the rate of diminution in clutch size is affected by the type of food.

The reduction in development time with increased temperature is greatest in the lower range with differences lessening at the higher temperatures. Similarly, Burgis (1970) reports, for Thermocyclops neglectus, a decrease in the effect of temperature changes with rise in temperature and summarizes similar reports by various authors for other species. An optimal temperature, above which development time is increased with increasing temperature, was not observed in the present experiments, although Burgis (1970) reports such an optimum for T. neglectus and other species.

While an optimal temperature for development time was not observed, the length of the complete egg production cycle does increase when the temperature exceeds an optimum level. The time from oviposition to oviposition is inversely related to temperature up to 27° incubation, but the cycle is longer at 31° incubation. Although embryonic development within the egg is not inhibited at 31°, some temperature-related maximum in the physiological process of egg production has been exceeded, with the result of an increased interval between clutches.

Aycock (1942) reported 48.5% of Cyclops vernalis nauplii incubated at 28.1° reached maturity, while only 7.84% reached maturity when incubated at 7.7°. In contrast, this paper reports an essentially inverse relationship for D. clavipes "native" to their incubation temperature between hatching success and temperature (the mean for 14° is slightly lower than the mean for 21°). Although Aycock's temperatures are quite different than ours, the data suggest the need for additional experiments to determine whether the two species differ with respect to the relationship between temperature and survival, or whether higher hatching success at the lower temperatures is balanced by higher mortality among nauplii.

Despite the fact that a 100% hatch is reported here rather infrequently (Appendices C-1, -2, -3, -4), it nevertheless seemed, as discussed previously, that in most cases either all eggs hatched or none at all did. Due to the experimental design, it was not possible to determine more accurately if hatching successes actually were greater than reported. However, Taub and Dollar (1968) found that in cultures of Daphnia pulex the frequency with which entire broods failed was greater than the frequency with which only a fraction of a brood failed. This is in agreement with what is suspected in the present study, although Taub and Dollar attribute the failures to inadequate food and this condition is not suspected in our cultures.

Using the results of this study (summarized in part in Table 25), the daily recruitment of nauplii per female at each temperature can be estimated. Multiplying hatching success by mean total number of eggs produced at each temperature shows (row A, Table 26) a decrease in total nauplii in the order 21°, 14°, 27°, 31°. This value, divided by the mean adult longevity of females at each temperature yields (row B, Table 26) the daily rate at which nauplii are added to the population at each temperature averaged over the adult lifetime of the individual. Row C, Table 26 (computed by multiplying mean clutch size by hatching success, then dividing by the rate of clutch production and converting from hours to days) lists the mean rate of addition to the population during the time that females are breeding. Both of these rates are highest at 27°, despite the reduced hatching success at that incubation temperature, and the maximum rate of recruitment appears to occur between 21° and 27°.

Table 25. SUMMARIZED RESULTS

Incubation temperatures (degrees C)	14	21	27	31
Adult longevity (days)	84.6	43.2	27.0	19.2
*Mean clutch size	26.8	28.0	30.0	21.3
*Mean total number of eggs per female	322.0	343.9	340.0	117.3
Mean rate of clutch production (hours)	166.7	63.7	47.3	55.2
Hatching success (%)	82.3	85.8	71.7	60.6

*Mean for females "native" to each incubation temperature.

Table 26. ESTIMATED PRODUCTION OF NAUPLII

Incubation temperatures (degrees C)	14	21	27	31
A. Total nauplii per female	272.4	295.1	243.8	71.1
B. Daily rate of production of nauplii averaged over adult lifespan	3.13	6.83	9.03	3.70
C. Daily rate of production of nauplii while female is actively breeding	4.54	9.05	10.91	5.61

SUMMARY

The effects of temperature and certain other factors on a number of the reproductive attributes of Diaptomus clavipes have been evaluated. The following conclusions have been reached:

1. The physiological longevity of adult females is inversely related to temperature.

2. There are some indications that the temperature at which a female hatches and spends her early immature stages is related to the size of the clutches she produces. It appears that, except near the upper thermal limit, the largest clutches are produced by females that have been at a constant temperature all their lives.

3. The temperature at which the female is kept also affects clutch size. This effect was noted primarily, however, with regard to the average clutch size being reduced at the highest temperature. The differences in average clutch size among females from 27, 21, and 14°C were relatively minor.

4. The total number of clutches produced by a female during her lifetime showed some indications of being related to temperature.

5. The number of clutches a female has produced previously does not have any large effect on clutch size.

6. Based on results from only three females, resting eggs are carried by the females for a shorter time than are subitaneous eggs, but the time after a clutch of resting eggs until a new clutch is produced is not different between the two types of eggs.

7. The temperature of hatching and early life does not seem to have any substantial effect on rate of clutch production.

8. The amount of time females carry their clutches is inversely related to temperature.

9. The interval between successive clutches is also related to temperature but with the shortest time between 21 and 27°C.

10. Hatching success is related to temperature with a maximum in the vicinity of 21°C.

CHAPTER 4

ASPECTS OF THE DYNAMICS OF A NATURAL POPULATION OF *DIAPTOMUS CLAVIPES*

Even with the ability to culture and experiment with an organism in the laboratory, it is still necessary to study naturally occurring populations. Controlled laboratory experiments provide a means of separating the effects of the many factors that influence a population, and thus they furnish a basis for interpreting the usually rather complicated population fluctuations found in nature. However, only from studies on field populations is it possible to determine whether the relations found in the laboratory hold in nature and, if so, which of the relations are of importance in actual environmental situations.

Thus, considering our overall goal of providing increased knowledge of the relation of diaptomids to water quality, a study on certain aspects of the population dynamics of a field population of *Diaptomus clavipes* was carried out. This work naturally fell into three broad categories: 1) heterogeneity of distribution, 2) a life table approach to population dynamics, and 3) reproduction. Separate sections dealing with the results and discussion for each of these aspects are included in this chapter after the Methods and Materials section.

A number of studies have dealt with some aspects of the relations between a diaptomid population and environmental factors. Such work was pioneered by G.W. Comita and coworkers (Comita, 1956; Comita and Comita, 1956; and Comita and Anderson, 1959) and by Ravera and Tonolli (Ravera, 1954, 1955; Tonolli, 1964, 1961; and Ravera and Tonolli, 1956). More recently,significant studies along these lines have included the work of Elster (1964), Armitage and Davis (1967), Healy (1967), Smyly (1968), Chapman (1969), and Kibby (1971).

METHODS AND MATERIALS

The study pond is located in Section 11, Township 9N, Range 3W of Cleveland County, Oklahoma. It is approximately 13.1 km SSE of Norman, Oklahoma. It is a man-made impoundment receiving runoff from the surrounding prairie at its east and southwest margins. There are no fish in the pond. The morphometry of the pond (Appendices D-1 and D-2) was determined following procedures outlined by Welch (1948). Pennak (1957) suggested that if one wants a reliable estimate of species composition and relative abundance of each species in an aquatic environment sampling from top to bottom is imperative. The same reasoning applies when determining the absolute numbers of a given species. In the present study sampling was carried out by means of a semi-pliable, 6.5-cm diameter, wire-embedded polyethylene tubing, suitably calibrated for depth measurements. A rope was attached to one end of the tube. This end was lowered perpendicular to the surface until it reached the bottom of the pond. After attaching a #20 plankton net over the opposite end of the tube, the lowered end was raised in a manner similar to that described by Pennak (1962), thereby causing a vertical column of water to be filtered through the plankton net.

Although sampling was carried out from May, 1970 until October, 1971, only data from 19 February through 29 October, 1971 are considered in this paper. These dates encompass the period of the year during which successful reproduction occurred in this population. On 19 February almost the entire population was in the adult stage and reproductive activity had begun, as evidenced by the high number of females carrying eggs. By the termination date, 29 October, although the temperature was still well within the range necessary for successful reproduction (approximately 10° to 30°C), successful reproduction was greatly curtailed. This was evidenced by the fact that only low numbers of immature copepodites were collected on the last three dates. Collections were taken every other day from 19 February to 20 April and every two weeks from 24 April until the termination of the study. The intensive collection period was instigated to determine the duration of the instars (Comita, 1956) and to allow the development of a horizontal life table (Deevey, 1947) for the first generation.

In analyzing reproduction it was the intent of this study, not only to follow and describe the various reproductive parameters, but also to relate variations in these parameters to temperature and food. Thus, at the same time that samples of animals were collected, the vertical temperature profile of the water was determined. Temperature readings were taken at 0.5-m depth intervals from surface to bottom in the open water region with a Whitney Underwater Thermistor.

Chlorophyll _a_ concentrations were used as an index of food availability. Water for chlorophyll _a_ determinations was gathered at 2-week intervals at the same time animals were collected. Five hundred milliliters of water was collected from both the surface and bottom of the open water region of the pond through use of a Kemmerer water sampler. The two samples were combined, the water was returned to the laboratory, and chlorophyll _a_ determinations were made following a modification of the procedures outlined by Small (1961). After shaking the water sample to mix it thoroughly two 200- or 300-ml subsamples were poured into separate 300-ml beakers. Which of these two amounts was used was determined by the turbidity of the water-- high turbidity, a 200-ml subsample, low turbidity, a 300-ml subsample. Each of the subsamples was passed through a membrane filter apparatus which contained a 0.8-micron (average pore size) filter to concentrate the phytoplankton. The filter was then removed from the apparatus and placed in a 50-ml test tube. The test tube was then capped. A duplicate procedure was followed using tap water rather than pond water in order to provide a control. Ten milliliters of 90% acetone were added to each test tube. The tubes were capped and placed in a constant temperature water bath (21°C) for 30 minutes. After 15 minutes the tubes were lightly shaken to aid mixing of the materials. When the 30-minute incubation period was over, the tubes were removed from the water bath; the dissolved materials were transferred to centrifuge tubes; and these were centrifuged at high speed for 1 minute. The supernate was then transferred to a cuvette, and, using the tap water extract to zero the spectrocolorimeter (B&L Spectronic 20), the percent absorbance of the two extracts at a wavelength of 665 millimicrons was determined. The average of two readings was recorded as a measure of the chlorophyll a present.

Field Data

To determine the dispersion pattern of the population, it was necessary to obtain data appropriate for statistical testing. During the preliminary period of sampling, it was apparent that most individuals of this species were located in open water rather than where rooted aquatics were growing. For this reason, the pond was divided horizontally into two regions or strata, the area where rooted aquatics came within 40 cm of the surface and the open water area. Eight samples were taken from each region of each date with the sites to be sampled selected by means of a random numbers table. The number of adults (copepodite VI) per liter in each sample was determined by dividing the total number of adults by the number of liters sampled.

The numbers of adult and copepodite V individuals of each sex, the number of females carrying eggs, and the numbers of the other copepodite stages were determined by complete census of each sample. The numbers of the various naupliar stages were determined after combining the samples into two groups, one from each stratum. Each pooled sample was then divided several times through use of a plankton splitter, and the nauplii in one of the resulting subsamples were counted. The mean number of eggs per clutch was determined at the same time. The number of splits to be carried out was chosen to ensure the census of at least 20 clutches. The amount of time an individual spends in naupliar stages I to III is relatively small (apparently less than 24 hours); and, for this reason, these stages were uncommon in the samples, and so they were lumped for counting. The results of all counts are presented in Appendices E-1 through E-8.

Laboratory Data

Animals were cultured in 100-ml beakers, each containing 80 ml of pond water which had been filtered twice through #20 bolting cloth. The beakers were kept in a constant temperature chamber at 21°C and under a daily cycle of 12 hours of light and 12 hours of dark. Twice weekly the animals were fed 0.1 ml of the aqueous mixture of trout food and alfalfa whose preparation has been described in Chapter 2. To initiate an experiment, a single pair of adult animals (one male and one female) was added to each beaker. The animals to be used were taken from a stock culture reared at the same temperature and light as used in the experiment and consisting of individuals at least one generation removed from the field.

Two questions pertaining to the effect of rooted aquatics on D. clavipes were studied in the laboratory. The first question concerned the ability of the adults to survive when they were forced to live in an environment in which Potamogeton sp., a "narrow leafed species" (Fassett, 1957), was allowed to float free. Two sets of 10 replicates each were used in this study. One set of beakers had Potamogeton sp. floating freely in the water, while the other set had no Potamogeton sp. added. To ensure that the results obtained were due to the vegetation rather than to periphyton, the Potamogeton sp. was soaked for 15 minutes in tap water and then for 15 minutes in distilled water. The vegetation was then washed with fast flowing tap water for 5 minutes after which it was placed in a container of double filtered pond water until it was used. After 4 days, during which time the beakers were censused daily for mortality, the experiment was terminated.

The second question studied in the laboratory concerned the effect of higher aquatics on reproduction. The female of D. clavipes generally carries her eggs in a sac on the underside of the last body region, or urosome, until they hatch into nauplii. We have found (see Chapter 3) that temperature affects the length of time the eggs are carried by the female of this species. A series of experiments was designed to determine if rooted aquatics also can affect the length of time eggs are carried and something of the nature of any effect that was found.

Five sets of 10 replicates each were used in the study. One set of beakers (designated weed-restricted) had pieces of *Potamogeton* sp. restricted to a small region with nylon netting, while in a second set only nylon netting was added. A third group of containers had 15 pieces of polyethylene tubing of approximately the same cross-sectional diameter as the *Potamogeton* sp. suspended vertically and randomly in each container. Suspension of the polyethylene tubing in the containers was accomplished by fastening a section of nylon netting over the top of the beaker to act as a guide for the sections of tubing. The polyethylene tubing was soaked in distilled water for 96 hours prior to the onset of the experiment. A fourth set of beakers also included polyethylene tubing, but it was restricted to the perimeter of the beaker. The final set of beakers, the controls, had nothing added.

In an attempt to keep conditions constant, animals were transferred weekly to a clean beaker containing fresh water. At this time young were removed and discarded. The beakers were checked at 24-hour intervals for animals carrying eggs and for nauplii. Checking for nauplii was necessary to ensure that a clutch had not been produced and then hatched during the preceding 24-hour period.

HETEROGENEITY OF DISTRIBUTION

Field Data

At the onset of the study the concentration of adults did not vary greatly between the two regions of the study pond—a higher concentration was noted in the open water on one date and in the region of rooted aquatics on the next (Figure 5). Starting with 6 April, however, a definite pattern, with a higher concentration of adults in the open water region than in the region of rooted aquatics, began (Figure 6). This pattern prevailed until 21 October. The concentration of adults ranged from 0.00 per liter in the area of rooted aquatics on 25 June to approximately 18 per liter in the open water region on 23 July.

A Student's t-test was conducted separately on the data from each sampling date to determine whether the concentration of adults varied significantly between the two regions. A detectable difference was found on only 5 of 23 dates prior to 6 April, i.e., 23 February; 9, 17, and 25 March; and 2 April (Figure 5), but from 6 April until 20 August, a significant difference ($P<0.25$) between means was found on all but 1 of 18 dates (Figure 6). This onset of relatively continuous stratification coincided with two observations, an increased rate of growth of rooted aquatics and an addition to the population of new adults which developed from the current year's reproductive activity (see Life Table Approach to Population Dynamics).

More evidence of differential distribution between the two areas of the pond was obtained when the proportion of the total water volume in the open water region and the fraction of animals collected in open water were both graphed against time (Figure 7). If the animals were not concentrating in one of the areas of the pond, the probability of one proportion being larger than the other on any given date would be 0.5. However, if the animals were concentrating in one of the areas, one of the proportions would be consistently larger than the other. Each data point was placed in two of three groups on the basis of the time of year it was obtained. The groupings were total sampling period (19 February to 29 October), early spring (19 February to 4 April), and spring-summer (6 April to 29 October). The early April date was chosen as the dividing time because of the increased rate of growth observed in the rooted aquatics from that date on and because the addition of new adults to the

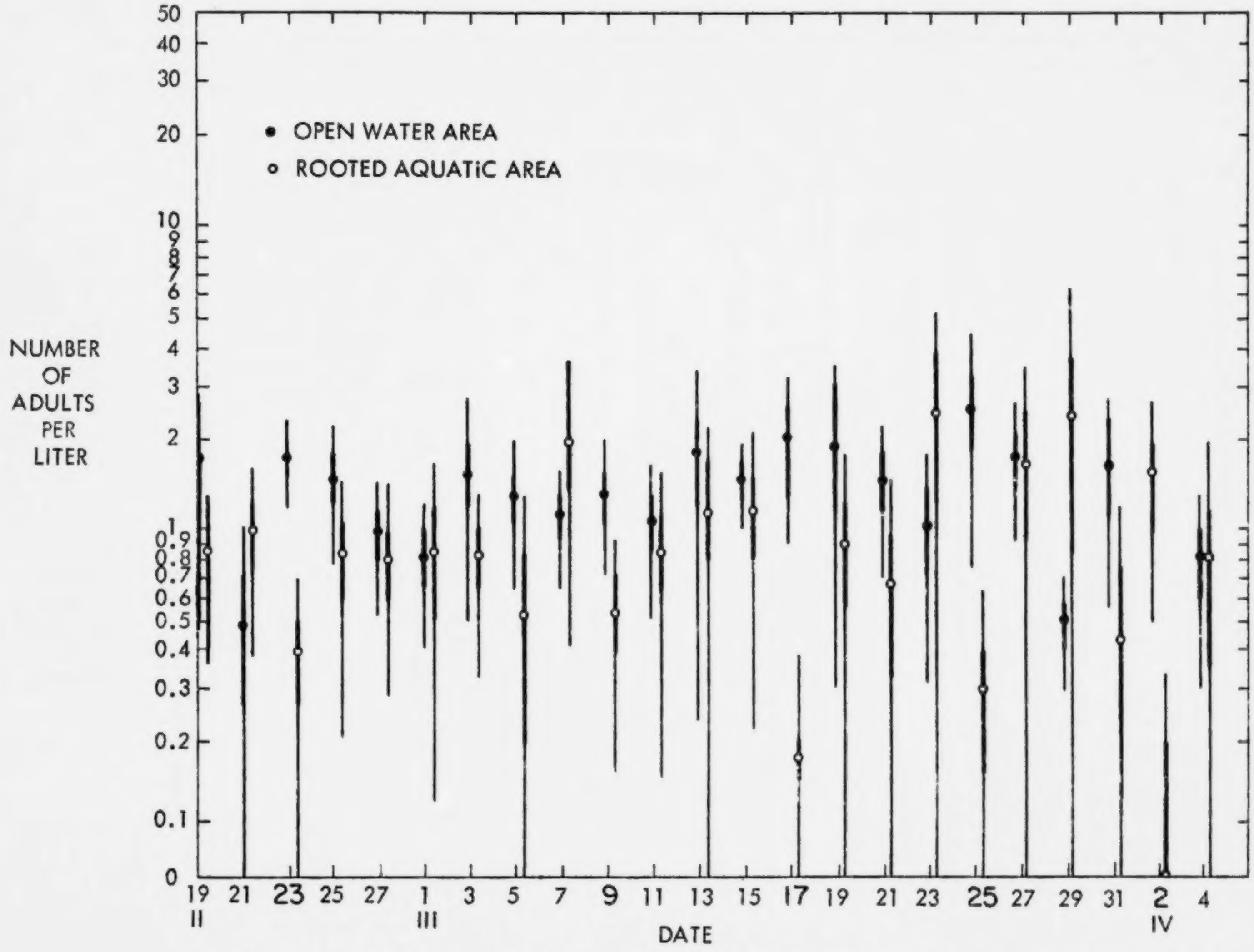

Figure 5. Mean Numbers of Adults Per Liter in the Two Regions of the Pond from 19 February to 4 April, 1971. Enlarged Arabic Numerals show those Dates when there were Detectable Differences ($P<0.05$) Between Concentrations of Adults in the Two Regions.

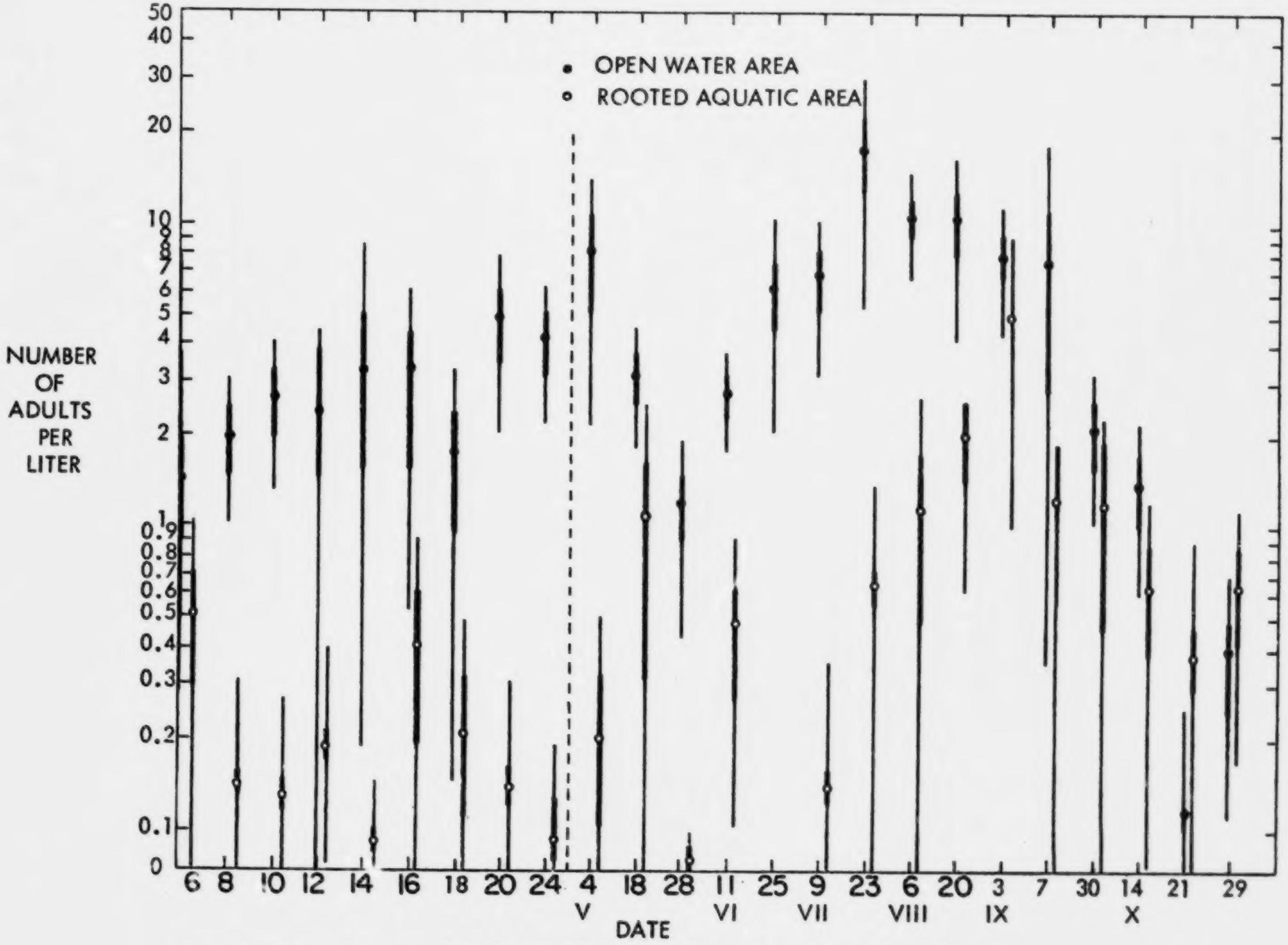

Figure 6. Mean Number of Adults Per Liter in the Two Regions of the Pond from 6 April to 29 October, 1971. Enlarged Arabic Numerals Show those Dates when there were Detectable Differences (P<0.05) Between Concentrations of Adults in the Two Regions. The Vertical Dashed Line Between 18 April and 24 April Shows a Change in Scale from 2-Day to Approximately 14-Day Intervals.

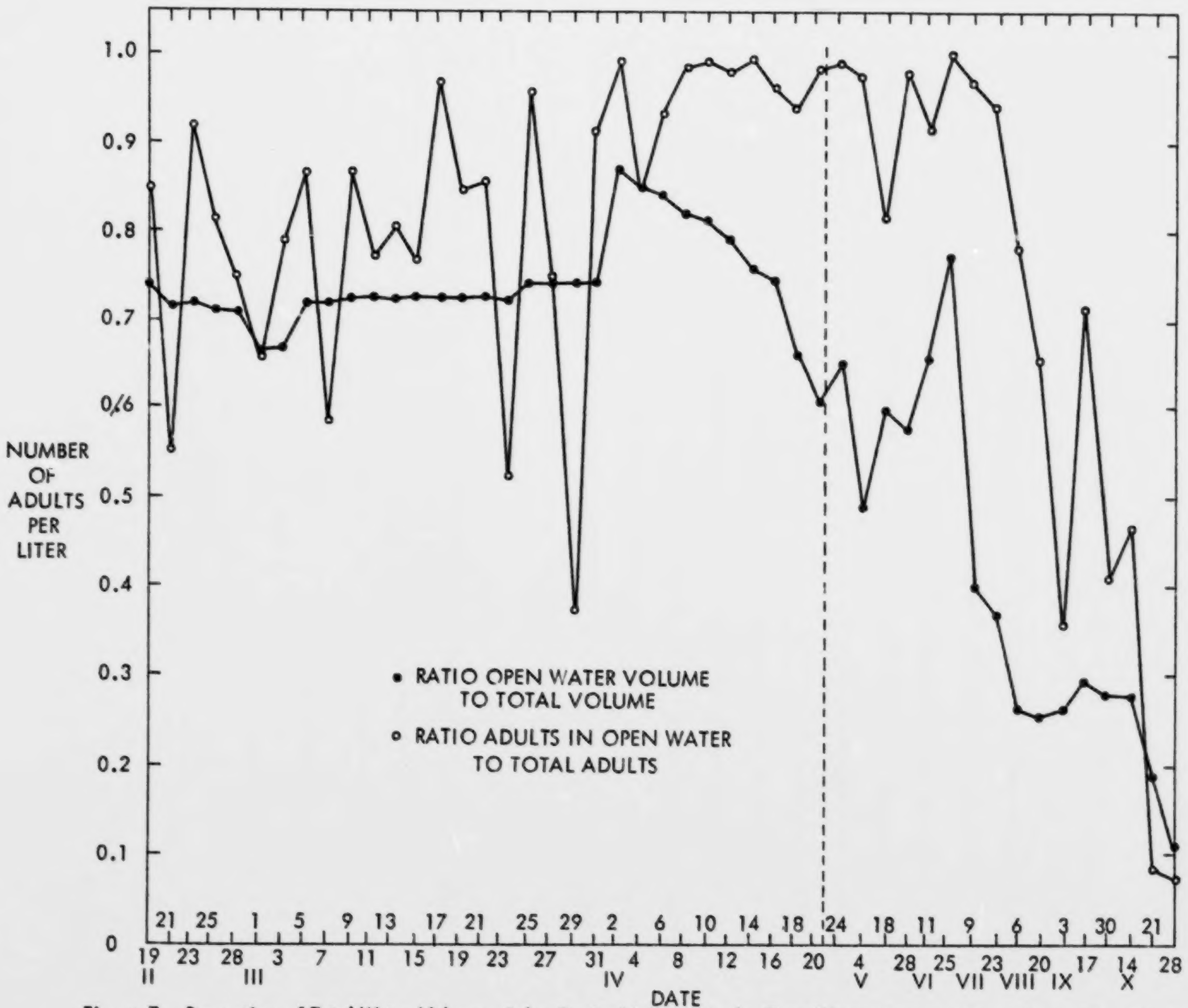

Figure 7. Proportion of Total Water Volume of the Pond which was in the Open Water Area and Proportion of the Total Number of Adults which were Collected in the Open Water Region on each Collecting Date. The Vertical Dashed Line Between 20 April and 24 April Shows a Change in Scale from 2-Day to Approximately 14-Day Intervals.

population began then. In approximately 75% of the collections during early spring and 88% of the collections during the spring-summer period, the proportion of the total adult population located in the open water region was greater than the proportion of the total water volume located in this region. These values are quite different from expected and are further evidence that the adult population is not evenly distributed between the two regions.

Although the Student's t-test showed a detectable difference in the concentration of adults in the two regions of the pond during most of the sampling dates during the spring-summer period, it did not show a similar pattern during the early spring period. Another method of testing for differences between the two regions in the early spring would be to run an F-test comparing all the data from the entire period in one test. That is determining whether there were significant differences in the early spring between the average number of adults per liter in the open water region and the average number per liter in the area in which rooted aquatics were found. When such a test was conducted, the F value was found to be significant at the 0.05 level (Table 27). The results of this test plus those of the t-tests described earlier show that significant differences existed between the concentrations of adults in the two regions of the pond throughout much if not all of the collecting period. In the early spring the differences were less pronounced and could be demonstrated for the entire period only, while in the spring-summer period they were stronger and were demonstrated for most of the individual sampling dates.

Table 27. AN ANOVA TEST COMPARING THE MEAN NUMBER OF ADULTS PER LITER IN THE OPEN WATER REGION AND THE MEAN NUMBER IN THE REGION IN WHICH ROOTED AQUATICS WERE FOUND DURING THE EARLY SPRING (19 February to 4 April, 1971).

Source of variation	df	DD	MS	F
Among regions	1	2.2751	2.2751	7.1454*
Within regions	44	14.0138	0.3184	
Total	45	16.2889		

*P < 0.05.

Laboratory Data

The field data revealed heterogeneity of distribution prevailed throughout the collecting period and appeared to be correlated with the growth of higher aquatics. The laboratory experiments discussed below showed that these differences in concentrations were, in fact, a result of the rooted aquatics.

In an experiment where 20 animals were forced to live in environments in which pieces of Potamogeton sp. were floating freely, 18 died within 96 hours; whereas of 20 animals in a comparable environment without Potamogeton, only one died in the same interval. This experiment clearly demonstrated that adult D. clavipes are unable to survive when forced to live in proximity to Potamogeton sp.

Comparison of the mean clutch carrying times for females in the simulated-weed and weed-free environments revealed that those animals in simulated weeds carried their eggs almost twice as long as did the animals in weed-free environments, 4.00 days and 2.22 days respectively (Table 28). As a check on these results, the environments of the animals were reversed, with those animals previously in simulated-weed environments now in weed-free environments and those animals previously in weed-free environments now in simulated-weed environments. After the environments were switched, the mean clutch carrying times were 3.71 days in the simulated-weed environment and 1.86 days in the weed-free environment (Table 29), again almost twice as long in the simulated weeds as in the weed-free environments. In both cases the results were highly significant ($P < 0.01$) when the Student's t-test was employed. Comparison of the mean clutch carrying times for females in the weed-free environments and the environments having polyethylene tubing around the perimeter of the beakers (Table 28) showed little difference between the two. This indicated that the difference between the average clutch carrying time for animals in simulated-weed environments and that for animals in weed-free environments was not due to some chemical found in the polyethylene tubing. This work showed that simulated weeds increase

Table 28. MEANS ($\bar{Y}$) AND STANDARD ERRORS ($S_{\bar{Y}}$) FOR THE TIMES EGGS WERE CARRIED BY FEMALES IN SIMULATED WEED ENVIRONMENTS, WEED-FREE ENVIRONMENTS, AND ENVIRONMENTS IN WHICH THE SIMULATED WEEDS WERE RELEGATED TO THE PERIMETER OF THE BEAKER.

Environment	n	$\bar{Y}$ (days)	$S_{\bar{Y}}$	P
Simulated-weed	18	4.00	0.52	< 0.01
Weed-free	18	2.22	0.22	
Simulated weeds relegated to the perimeter of the beaker	18	2.01	0.25	

Table 29. MEANS ($\bar{Y}$) AND STANDARD ERRORS ($S_{\bar{Y}}$) FOR THE TIMES EGGS WERE CARRIED BY FEMALES IN SIMULATED WEED ENVIRONMENTS AND WEED-FREE ENVIRONMENTS AFTER THE ENVIRONMENTS OF THE ANIMALS WERE REVERSED.

Environment	n	$\bar{Y}$ (days)	$S_{\bar{Y}}$	P
Simulated-weed	7	3.71	0.42	< 0.01
Weed-free	7	1.86	0.14	

the length of time eggs are carried by the females thus suggesting that the effect of rooted aquatics on the copepods is partially due to the actual physical presence of the plants. The cause or causes of this developmental retardation and the pathway by which it is implemented are not known and deserve further investigation.

A comparison of the mean clutch carrying times for animals kept in restricted *Potamogeton* sp. environments and nylon-netting environments (Table 30) revealed no significant difference.

Table 30. MEANS ($\bar{Y}$) AND STANDARD ERRORS ($S_{\bar{Y}}$) FOR THE TIMES EGGS WERE CARRIED BY FEMALES IN ENVIRONMENTS IN WHICH *POTAMOGETON* SP. WAS RESTRICTED TO A SMALL REGION BY NYLON NETTING AND IN NYLON-NETTING ENVIRONMENTS.

Environment	n	$\bar{Y}$ (days)	$S_{\bar{Y}}$	P
Weed-restricted	6	2.66	1.09	0.90
Nylon-netting	6	3.00	0.45	

Field data revealed a heterogeneous distribution of *D. clavipes* during the entire study period apparently related to the occurrence of rooted vegetation. Laboratory data showed that rooted aquatics could cause the death of the copepods and that even the presence of simulated weeds caused retardation of development and hence presumably a decrease in reproductive rate. Apparently the rooted aquatics are an effective factor in determining the regions of a body of water in which this species can live and reproduce.

In the past, investigations of zooplankton in small bodies of water have commonly utilized one or two tows of a plankton net to determine species composition and abundance. In using such data to draw conclusions concerning the populations in the pond, it has usually been assumed that a relatively homogeneous environment existed in the pond such that the species present were either randomly or uniformly distributed. The data from the current study demonstrate that this assumption is not necessarily correct, at least if macrophytes are present near the surface.

LIFE TABLE APPROACH TO POPULATION DYNAMICS

During February and early March 1971, false starts in the population development occurred during periods of relatively warm weather (surface water temperature between 6° and 9°C). Eggs were produced and hatched, and the nauplii developed to naupliar stage IV at which point development ceased and the individuals eventually died. Data from these individuals were not used in determining the durations of the various instars. Since only adults and a few early nauplii were found at the start of this investigation, the duration of each immature instar was estimated as the length of time between the first observation of the instar in the population and the first observation of the succeeding instar. This procedure could not be used in estimating the duration of the adult instar, however, since at no time were adults absent from the population. Instead, the duration of the copepodite VI stage was estimated by measuring the length of time between successive minima in the numbers of adults in the population. The minima in adult numbers were assumed to result from periods high mortality in this instar group. A minimum occurred on 18 April 1971 with another one occurring on 28 May 1971 (Figure 8). The period of time between these two dates, 40 days, was taken as an estimate of duration of CVI. This agrees closely with the estimate of 43.2 days obtained for the duration of the adult female life span in the laboratory at 21°C (Table 15). The mean water temperature in the field during this period was between 15° and 20°C. The value of the mean temperature in this context is debatable, however, since vertical migration of the individuals and the differing temperature patterns at the various water depths would enable the organisms to live in a variety of temperature situations. The exact correlation of instar

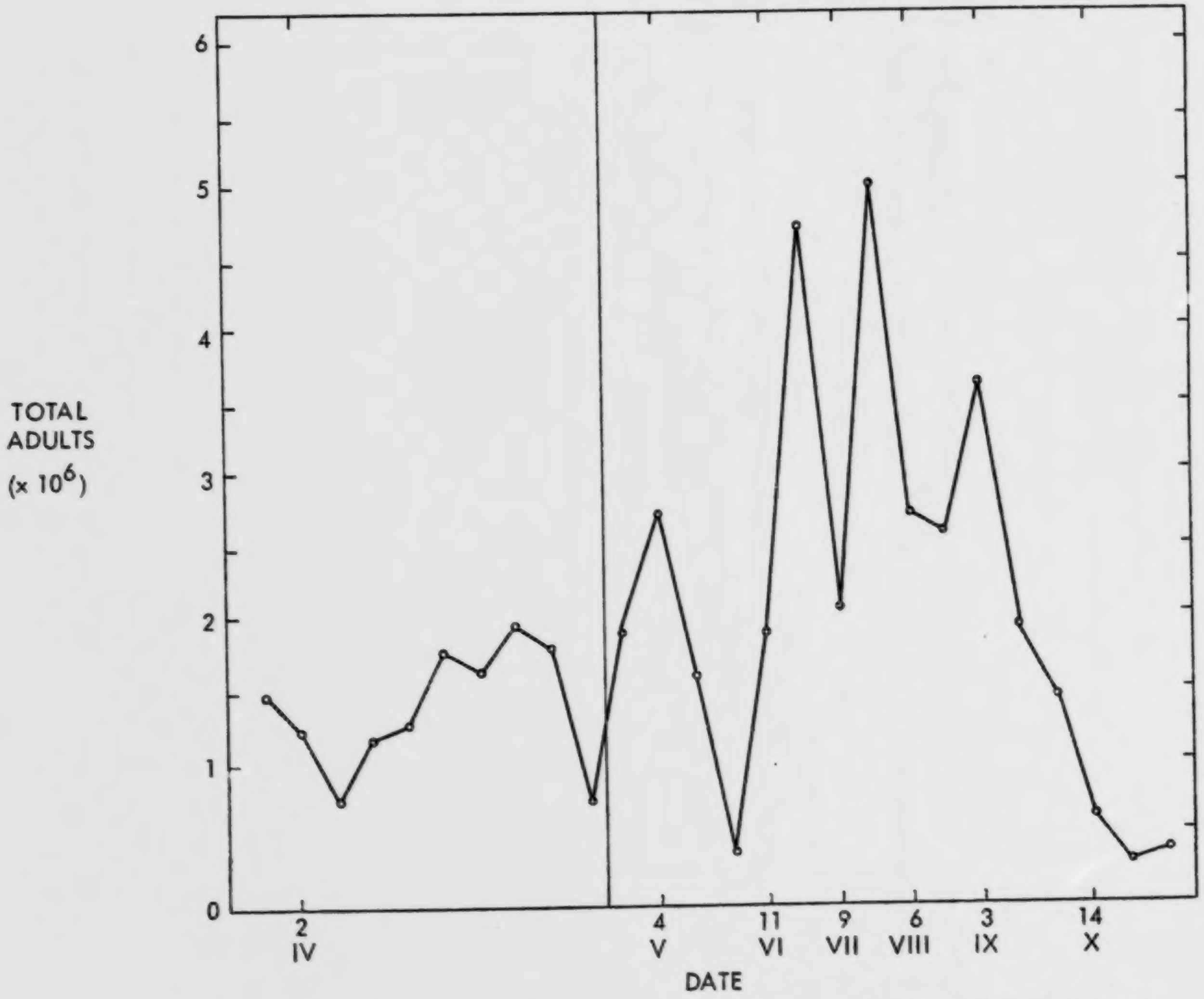

Figure 8. Size of the Adult Population from 2 April to 29 October. The Vertical Line Between 18 April and 24 April Shows a Change in Scale From Two Day to Approximately Fourteen Day Intervals.

durations with temperature is further complicated by food, since both quantity and quality of food apparently affect the developmental rates of copepods (Coker, 1933; Smyly, 1970).

Table 31 shows the durations of the various instars as determined in the field and also as computed from a composite of laboratory data. The laboratory data are from Samples (1972) and were obtained within a temperature range of approximately 20°C to 25°C. Several studies have shown an inverse relationship between temperature and the developmental time of the various instars, for example McLaren (1965) found a longer developmental time in the Arctic calanoid Pseudocalanus at lower temperatures than at higher ones. When the field durations (obtained at lower temperatures) are compared to the laboratory durations, similar results are noted for D. clavipes with a total developmental time to CVI of 28 days in the field and 21.5 days in the laboratory.

Life tables were constructed for the first generation (g_1) (Table 32), total year (Table 33), and laboratory animals (Table 34). In computing the life tables, the durations based on the laboratory results were used except for the first generation (g_1). This procedure was justified for the field population on the basis that during the reproductive part of the year, except during g_1, the pond temperatures were generally within the range 15° to 25°C (see Figure 14, page120), and the animals probably migrated within the pond in order to avoid higher temperatures when they did occur. Thus, the durations from the laboratory for temperatures in the range 20° to 25°C were assumed to represent a reasonable first approximation for the instar durations during the generations after g_1. Since the field durations were directly determined for the first generation,these were used for developing the life table for this generation.

The number of individuals entering each of the various stages for the g_1 and laboratory study were determined by following the survival of a cohort of individuals at close intervals. This was accomplished by computing the loss of individuals in passing from

Table 31. DURATIONS OF THE VARIOUS INSTARS AS DETERMINED IN THE FIELD AND COMPUTED FROM A COMPOSITE OF THE LABORATORY DATA (approximately 20° to 25°C; Samples, 1972).

Stage	Duration	
	First Occurrence	Laboratory
Egg-NIII	2	1.8
NIV	2	1.4
NV	2	1.2
NVI	2	1.2
CI	2	1.5
CII	2	1.9
CIII	6	3.5
CIV	6	4.4
CV	4	4.6
Composite through CV	28	21.5
CVI	40	40.00 (used field duration after comparison with Table 15)
Total life cycle	68 days	61.5 days

Table 32. LIFE TABLE FOR THE FIRST GENERATION USING THE FIRST OCCURRENCE TO DETERMINE THE DURATIONS OF THE VARIOUS STAGES.

Stage	Number living at beginning of age interval 1_x	Number dying in interval d_x	Mortality rate per 1000 alive at beginning of age interval q_x	Mean number alive L_x	Total life expectancy T_x	Mean lifetime remaining for those attaining age interval e_x
Egg-NIII	1000.00	836.62	836.62	581.69	3119.02	3.12
NIV	163.38	17.22	105.39	154.77	1955.64	11.97
NV	UNABLE TO DETERMINE FROM DATA.					
NVI	146.16	89.73	613.87	101.30	1336.56	9.14
CI	56.44	1.05	18.61	55.92	1133.96	20.09
CII	55.39	15.79	285.07	47.49	1022.12	18.45
CIII	39.60	16.74	422.73	31.58	927.14	23.41
CIV	22.86	0.57	24.93	22.58	739.76	32.36
CV	22.29	-3.16	-141.63	23.87	604.28	27.11
CVI	25.44	25.44	1000.00	12.72	508.80	20.00

Original 1_x equals 10.454×10^6.

Table 33. LIFE TABLE FOR THE COMPLETE YEAR STUDY USING THE COMPOSITE LABORATORY DATA TO DETERMINE THE DURATIONS OF THE VARIOUS STAGES.

Stage	Number living at beginning of age interval 1_x	Number dying in interval d_x	Mortality rate per 1000 alive at beginning of age interval q_x	Mean number alive L_x	Total life expectancy T_x	Mean lifetime remaining for those attaining age interval e_x
Egg-NIII	1000.00	881.40	881.40	559.30	1691.90	1.69
NIV	118.60	50.62	426.79	93.29	679.57	5.73
NV	67.98	15.24	224.15	60.36	549.89	8.09
NVI	52.74	24.76	469.42	40.36	477.46	9.05
CI	27.98	1.22	43.54	27.38	427.41	15.28
CII	26.77	11.09	414.21	21.22	387.17	14.46
CIII	15.68	0.85	53.93	15.26	347.06	22.13
CIV	14.83	0.18	11.80	14.75	294.26	19.84
CV	14.66	5.88	401.01	11.72	230.10	15.70
CVI	8.78	8.78	1000.00	4.39	175.60	20.00

Table 34. LIFE TABLE FOR LABORATORY ANIMALS USING COMPLETE DATA FROM ALL ANIMALS AT ALL TEMPERATURES.

Stage	Number living at beginning of age interval 1_x	Number dying in interval d_x	Mortality rate per 1000 alive at beginning of age interval q_x	Mean number alive L_x	Total life expectancy T_x	Mean lifetime remaining for those attaining age interval e_x
Egg-NIII	1000.00	281.51	281.51	859.29	12917.72	12.92
NIV	718.49	115.55	160.82	660.71	11362.49	15.81
NV	602.94	71.43	118.47	567.23	10444.11	17.32
NVI	531.51	151.26	284.58	455.88	9763.43	18.37
CI	380.25	18.91	49.72	370.80	9198.14	24.19
CII	361.34	35.71	98.84	343.49	8653.06	23.95
CIII	325.63	27.31	83.87	311.97	8003.87	24.58
CIV	298.32	18.91	63.38	288.87	6924.45	23.21
CV	279.41	54.62	195.49	252.10	5667.87	20.29
CVI	224.79	224.79	1000.00	112.39	4495.60	20.00

Original 1_x equals 1.302×10^3.

one stage to the next. In g_1 the number of individuals reaching CVI was estimated by multiplying the ratio of new adults to total adults by the total adult population on 31 March, the first date new adults were observed in the population. The g_1 adults were distinguished from older animals by their more opaque appearance and slightly smaller size. Prior to 31 March none of the adults collected were opaque. This opaque appearance was possibly related to the new carapace (following the molt from CV to CVI) not yet having hardened, although the cause was not determined. The total number of individuals entering each instar during the different generations or periods was determined by using the following formula:

$$N_i = \sum_{x=j}^{k} \frac{1_{i,x} + 1_{i,x+1}}{2} \frac{W_x}{D_i}$$

where: 1 refers to the number of individuals alive;

i refers to the instar designation;

x refers to the collection designation;

j refers to the first collection prior to the appearance of instar i;

k refers to the collection following the last collection in which instar i appears;

D_i refers to the duration in days of instar i;

W_x refers to the interval in days between collection x and collection x + 1;

N_i refers to the number of individuals of instar i produced in the interval x to x + 1; it also refers to the number of individuals of instar i produced in a particular generation.

Table 32 shows the life table for g_1. The 1_x and d_x columns of this life table reveal over 80% mortality occurred within the egg to NIII stage, with 836.62 out of every 1,000 individuals that entered the egg stage dying before reaching NIV. (Technically some animals undoubtedly reached NIV but died before being counted. To simplify the presentation in this section all the mortality recorded for a stage will be assumed to have occurred between the time of counting and the transformation to the next stage.)

Except for the adult instar which, of course, had a mortality rate (q_x) of 100%, the egg to NIII stage had the highest mortality rate. The second highest mortality rate occurred for stage NVI with 613.87 out of every 1,000 individuals dying during this interval. The CIII stage had the third highest rate, 422.73 per 1,000. The observation of an apparently negative mortality rate for stage CV is due to the inaccuracies of our population estimates. An overestimate of the numbers of CVI, an underestimate of the numbers of CV, or some combination of these two would lead to such a result. The apparently negative rate does suggest that mortality was low for stage CV.

The shortest life expectancy during g_1 was in the egg to NIII stage, with the remainder of the naupliar stages also having lower e_x values than any of the copepodite stages (Table 32). The longest life expectancy in this generation (32.36 days) was found in the CIV, although in all copepodite stages except CII (e_x of 18.45 days) the life expectancy was greater than 20 days. The life expectancies of the six copepodite stages contrast with the shorter expectancies of 3.12 days, 11.97 days, and 9.14 days found for the egg to NIII, NIV, and NVI stages, respectively.

The survivorship, mortality, and longevity rates for the complete study (Table 33) reveal similar trends as those for g_1. The highest mortality rate, 881.40 per 1,000 individuals, occurred in the egg to NIII stage. This was followed by a mortality rate of 469.42 per 1,000 for the NVI stage. The greatest life expectancy (22.13 days) was found in the CIII stage. Although life expectancies for the various stages of the complete year's data were not as long as those for the first generation, they followed a similar trend with all the copepodite stages having longer life expectancies than any of the naupliar stages. The lowest life expectancy was again in the egg to NIII stage, 1.69 days, while NIV, NV, and NVI had life expectancies of 5.73 days, 8.09 days, and 9.05 days, respectively. The lowest life expectancy among the copepodite stages was found for CII (14.46 days), with each of the other copepodite instars having a life expectancy of greater than 15 days.

The life table from the full year furnishes further evidence that the duration estimated for the adult stage is relatively accurate. A successful population having an annual reproductive period, no emigration or immigration, and numbers that are in part determined by the cycle of available food should have approximately the same number of individuals alive at the beginning of the reproductive period in one year as the next, provided that the available environment remains the same. The intrinsic rate of natural increase (Birch, 1948), or r, for such a population would be expected to approach 0 for the complete year. When r was calculated from the full year's data using a 40-day duration for the adult stage and the average m_x (number of eggs/adult/day) value for the year (see Figure 10, page 108), the result obtained was -0.03 (Table 35), surprisingly close to 0 in view of the limitations of the method employed.

The life table prepared from laboratory data (Table 34) shows that the mortality rates of the egg to NIII stage and the NVI stage were similar, with 281.51 and 284.58 deaths per 1,000 individuals, respectively. The mortality rates for the early stages were higher than those for the copepodite stages except CV. The mean life expectancies for the copepodite stages were all higher than those for the early stages, with values ranging from 20.00 days for the CVI instar to 24.19 days for CI and 24.58 days for CIII. The life expectancies for the early stages ranged from a low of 12.92 days for the egg to NIII stage to 18.37 days for the NVI instar.

Comparison of the three life tables reveals several interesting points. Although the three tables have similar trends in their mortality rates with the egg NIII and the NVI stages having the highest mortalities, the table derived from laboratory data shows almost the same mortality rate in the egg to NIII as in the NVI stage; whereas, in the tables derived from field results, mortality is far greater in the egg to NIII stage than in the NVI stage. Another difference between laboratory and field derived mortality is in degree. Approximately 28% of the eggs perished in the laboratory, while over 84% of all the eggs produced in the field and almost 84% of the eggs produced by the overwintering females failed to develop to NVI.

Table 35. CALCULATION OF THE INTRINSIC RATE OF NATURAL INCREASE (r) USING THE ADULT SURVIVORSHIP FROM THE FULL YEAR STUDY, A 40-DAY DURATION FOR THE ADULT STAGE, AND THE AVERAGE m_x VALUE FOR THE YEAR.

Stage	Duration	l_x	m_x	$l_x m_x$	$x l_x m_x$
Egg	0-1.81	1.00000	00.00		
CV	21.46				
CVI	25.46	0.00878	2.78339	0.02443	0.62980
CVI	29.46	0.00878	2.78339	0.02443	0.71970
CVI	33.46	0.00878	2.78339	0.02443	0.81742
CVI	37.46	0.00878	2.78339	0.02443	0.91514
CVI	41.46	0.00878	2.78339	0.02443	1.01286
CVI	45.46	0.00878	2.78339	0.02443	1.11058
CVI	49.46	0.00878	2.78339	0.02443	1.20830
CVI	53.46	0.00878	2.78339	0.02443	1.30602
CVI	57.46	0.00878	2.78339	0.02443	0.40374
CVI	61.46	0.00878	2.78339	0.02443	1.50146
		Total		0.24430	10.61720

T (generation time) = 39.45968

R_o (net reproductive rate) = 0.24430; ln = -1.4094

r = -0.03572

Figure 9 provides plots of the l_x curves for the first generation, laboratory population, and complete year's data thus allowing visual comparison of survivorship for the three groups. A definite similarity in form exists among the three curves with the greatest survival occurring in the laboratory population. If the survivorship curve of the laboratory population is considered to show the survival for the various stages without the effects of a natural environment, then the differences between this curve and the two field derived curves can be considered as rough indications of the effects of environmental factors.

The forms of the three survivorship curves range from a near diagonal curve for the immature stages of the laboratory animals (death occurring independent of age) to the positively skewed curve for the instars of the two field populations (high early mortality). It appears that a negatively skewed curve characterizes the adult stage of each group with those animals reaching adulthood surviving until they die of old age. This idea is supported by three pieces of evidence:

1. The adult population underwent relatively sharp declines in numbers, e.g., on 18 April and 28 May, which are assumed to have been the result of more or less synchronous death of a large segment of the adult population;
2. The r value calculated for the entire year was found to be close to 0.0 when a 40-day duration was assumed for the adult instar (Table 35);
3. The laboratory results show a life span close to that calculated from the field work (Table 15).

Life tables are valuable aids in determining general characteristics of animal populations. They also aid the investigator in determining those stages of the life cycle which have the highest mortality rates. This is of value to the individual studying pest species in that he can, through use of this approach, determine the weak points of the life cycle; and he can concentrate on increasing the mortality rates in these stages. If one is interested in studying the population dynamics or productivity of a

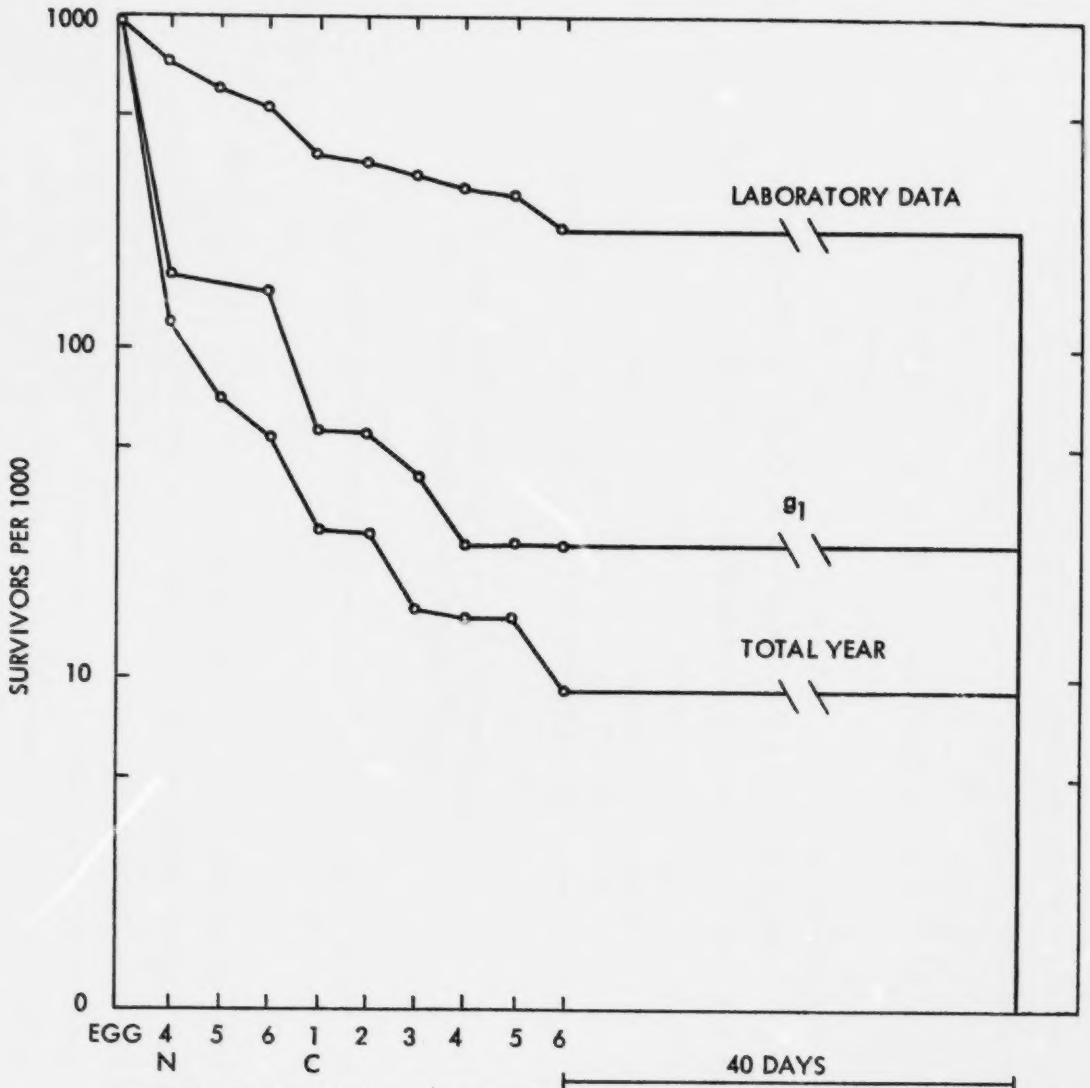

Figure 9. Survivorship Curves for the First Generation (g_1), Laboratory Population, and Complete Year's Data.

species of animal, the use of 1_x curves gives the information necessary in determining where to concentrate and what to study. In D. clavipes it is apparent that the cause of mortality (what) is of importance primarily during the egg to NIII interval (where) with the possible inclusion of the metamorphosis from NVI to CI. Mortality seems of little consequence in the adult instar, since a physiological 1_x curve is apparently present in this stage. Consequently with the adults,population studies should focus on those factors which may affect the reproductive rate.

REPRODUCTION

Analysis of the survivorship curves suggested three points of the life cycle for special consideration--egg production, survivorship from egg to NIII, and survivorship of NVI to CI. In this section consideration will be given to egg production and its relation to certain environmental factors.

Edmondson (1960) used as his reproductive index the ratio of eggs to animals in the population (crude birth rate). Since with D. clavipes the eggs are carried by the females and the adults are easily distinguished from the immature copepodite stages (Kamal and Armitage, 1967), it was possible to determine the ratio of eggs to adults in our work. Because the egg development time varies only slightly with temperature (Table 21) except near the lower limit for reproduction, a 2-day duration for the egg stage has been used to compute the specific birth rates. By dividing the total number of eggs by the product of the total adult population and the duration of the egg stage, a specific birth rate, or m_x value, can be calculated for any date.

An average (weighted for the differences in the sampling intervals) of 2.78 eggs per adult per day was calculated for the entire study period (Figure 10). During the first three collecting dates, when the temperature was well below the lower limit for reproductive activity as determined in the laboratory, the m_x values were below the yearly

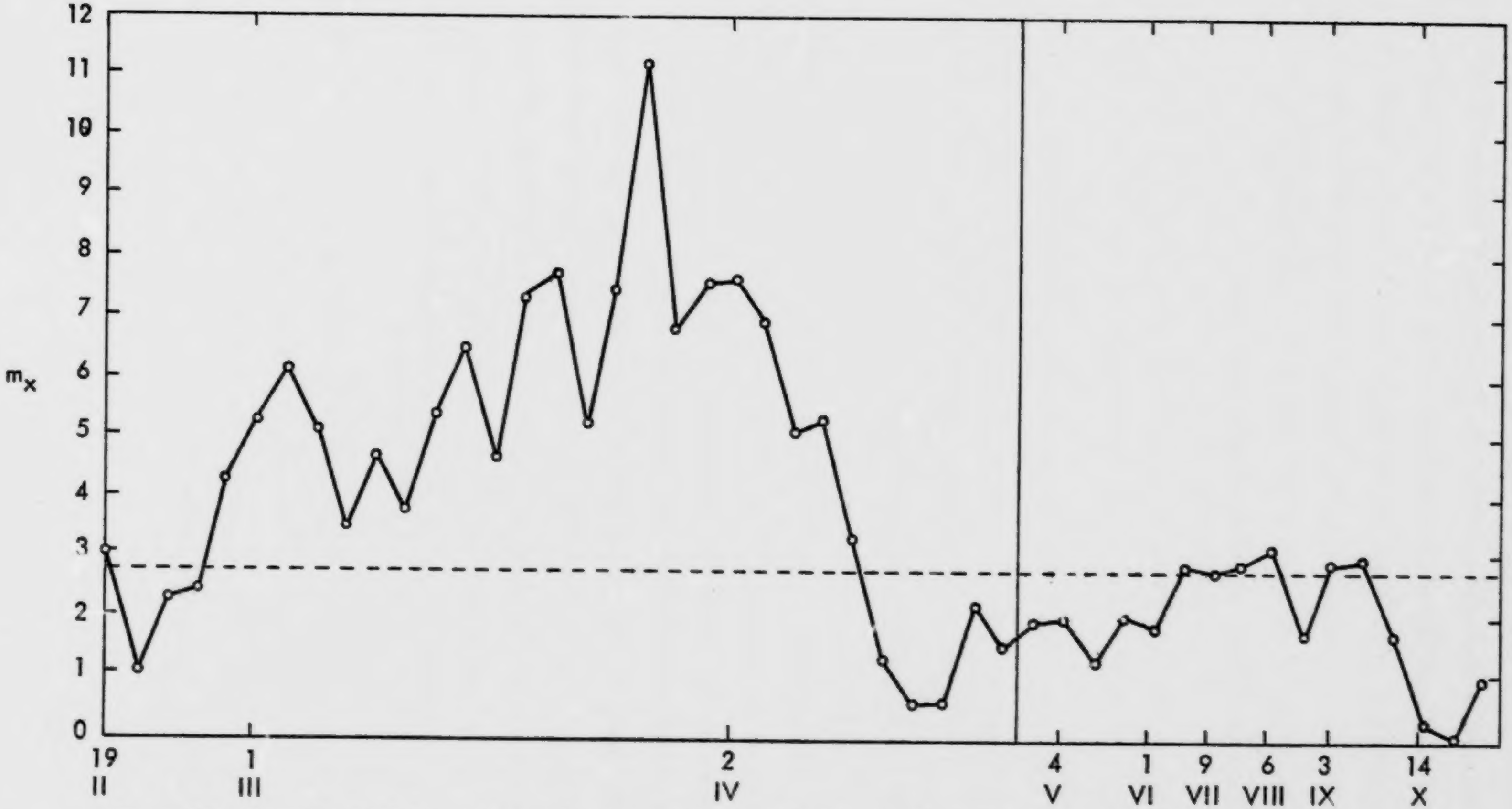

Figure 10. Specific Birth Rates (m_x) as Calculated for Each Collecting Date and the Weighted Mean m_x Value (2.78) for the Entire Reproductive Year. The Vertical Line Between 18 April and 24 April Shows a Change in Scale from 2-Day to Approximately 14-Day Intervals.

weighted mean. Starting with 21 February and continuing until 27 March, the m_x values tended to rise, peaking at a value over 9. After this date the m_x values steadily decreased but remained above the mean until 10 April. From this date until the culmination of the study, the m_x values rose above the mean only during the period of 9 July to 6 August and for a short time in September. The mean water temperature (see Figure 14, page 120) tended to rise during the period from 27 March until 3 September and was above 20°C for much of the summer. Our results in Chapter 3 indicated that reproductive rate increases with temperature to 25°C or somewhat higher. Thus, the seasonal variations in m_x values did not show the pattern expected from the temperature variations.

To determine whether the apparent differences in m_x values during the various periods of the year were statistically different, the data were divided into four groups by season. The first group of data, called the winter data, included the m_x values obtained from samples collected from the onset of the study, 19 February, until 31 March, when the first new individuals reached maturity. The second or spring group of m_x values were those from samples collected between 31 March and 28 May. This latter date was chosen because it was assumed to be the final die-off date for the first generation. The summer group included the values obtained for samples from 28 May until 30 September, while the fall group included values obtained from 30 September until 29 October. The fall season was characterized by decreasing temperatures, although they were well above the lower limit for reproduction.

Analysis of variance of the four group (Table 36) showed a significant difference among the means of the various seasons. To determine which pairs of means were significantly different, a Student-Newman-Keuls (SNK) test (Sokal and Rohlf, 1969) was used. The lowest mean m_x value was that for the fall season, followed in increasing order by the summer, spring, and then winter means (Table 37). The SNK test detected a significant difference between the members of all pairs of means except the spring-summer one (Table 37).

Table 36. ANOVA TABLE TESTING THE DIFFERENCES AMONG THE m_x VALUES OF THE VARIOUS SEASONS.

Source of variation	df	SS	MS	F_s
Among seasons	3	109.6651	36.5534	8.7624*
Within seasons	42	175.3545	4.1701	
Total	45	285.0196		

*$P < 0.01$

Table 37. A POSTERIORI COMPARISON OF THE MEAN SEASONAL m_x VALUES USING THE STUDENT-NEWMAN-KEULS TEST.

			Fall	Summer	Spring	Winter
$\bar{Y}$			0.4472	2.5779	3.0306	5.3306
n			3	9	14	21
s^2			0.2583	2.7774	5.3713	5.3967
	$\bar{Y}$	n				
F	0.4472	3	--			
Su	2.5779	9	2.1307*	--		
Sp	3.0316	14	2.5844*	0.4537	--	
W	5.3306	21	4.8834*	2.7527*	2.2990*	--

*$P < 0.05$

Three factors interacted to produce the specific m_x values observed; i.e., the percent of adult females carrying eggs, the ratio of males to females in the population, and the mean number of eggs per clutch on each date. As indicated in Figure 11, at least 50% of the adult females were carrying eggs during the greater part of this study. The only extended periods when less than 50% of the females were carrying eggs occurred from mid-April to mid-May and from mid-October until the culmination of the study on 29 October. The latter period coincided with a steady decrease both in water temperature and total adult population.

Although the average percent of the females carrying eggs appeared to differ among the four seasons, the variance was so great within the groups that analysis of variance showed no significant differences among the seasonal means (Table 38). It appears, therefore, that variation in the percentage of females carrying eggs was not a major determining factor for the seasonal variations of the m_x values that were noted in this study.

Table 38. ANOVA TABLE COMPARING THE MEAN PERCENTAGES OF FEMALES CARRYING EGGS DURING THE VARIOUS SEASONS.

Source of variation	df	SS	MS	F_s
Among seasons	3	74.1164	24.7054	2.3181*
Within seasons	43	458.2621	10.6572	
Total	42	532.3785		

*$P < 0.10$

Several investigators, including Chapman (1969), have suggested that, for certain copepods, males have a shorter life span than females. Elgmork (1959) found evidence to suggest that the males develop somewhat more quickly in Cyclops strennus strennus

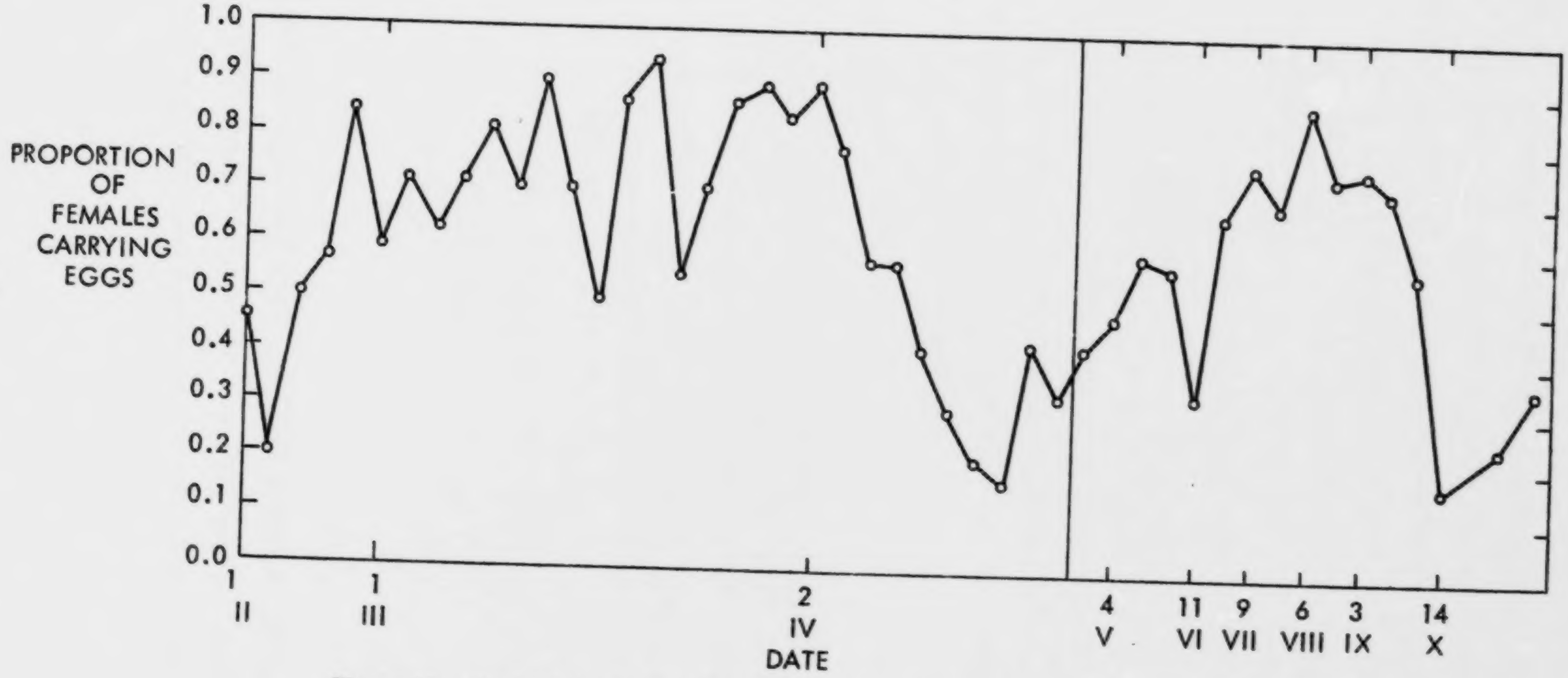

Figure 11. Proportion of Adult Females Carrying Eggs on Each Collecting Date. The Vertical Line Between 18 April and 24 April Shows a Change in Scale from 2-Day to Approximately 14-Day Intervals.

than the females, and thus males predominated during the early stages of several increases in the numbers of adults in his populations. Interpretation of the ratio of males to females on the various collecting dates in our study (Figure 12) suggests that males appeared before females in the various generations of this population also. This conclusion was reached from an examination of the ratio of males to females during the low periods of adult population numbers. On both 18 April and 28 May when the numbers in the total population were low (Figure 8), the ratio of males to females was significantly above the yearly average of 1.28 (tested by a modification of the Student's t-test designed to test the difference between a single observation and the mean of a group; Sokal and Rohlf, 1969). Previously it was indicated that these dates were taken as the die-off dates for the previous generations (winter and g_1, respectively).

An analysis of variance performed on the values for the mean ratios of males to females during the four seasons (Table 39) revealed a significant difference among the seasonal means. However, when an SNK test was run on these data (Table 40), the only seasonal differences detected were between the fall mean and those for each of the remaining three seasons. Since the ratio of males to females in the fall would have no bearing on the m_x values obtained during the preceding reproductive year, the ratio of males to females was assumed to be unimportant in determining the m_x values obtained in this study.

The final factor studied was the mean number of eggs per clutch (Figure 13). Analysis of variance showed a significant difference among the means for the four seasons (Table 41). The lowest mean number of eggs per clutch (15.25) occurred in the summer period, with the means for fall, spring, and winter following in ascending order (Table 42). The mean value for winter was twice that for summer, with 30.53 eggs per clutch occurring. A detectable difference occurred between the members of each pair of means except the summer-fall pair (Table 42). The mean number of eggs

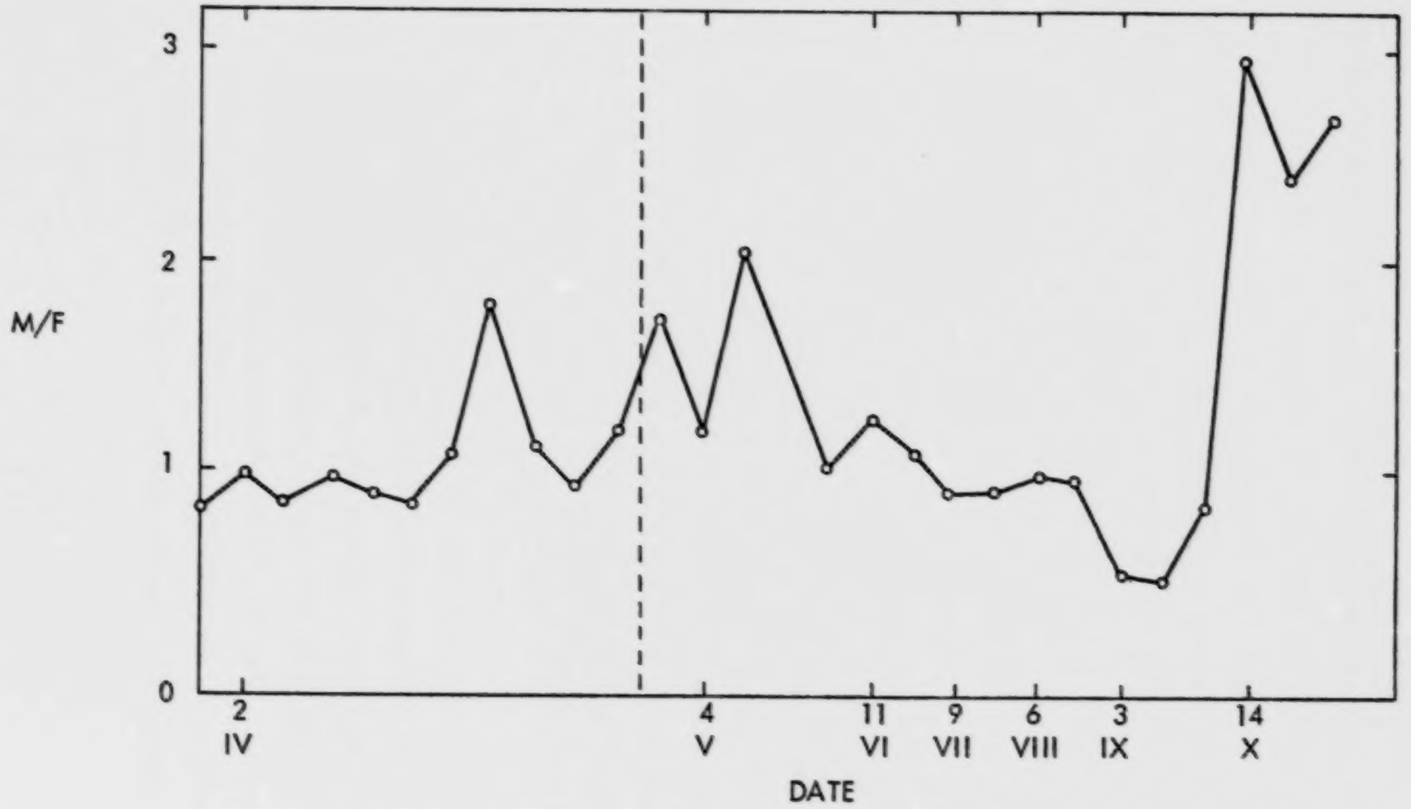

Figure 12. Ratio of Males to Females in the Adult Population on certain Collecting Date. The Vertical Dashed Line Between 18 April and 24 April Shows a Change in Scale from 2-Day to Approximately 14-Day Intervals.

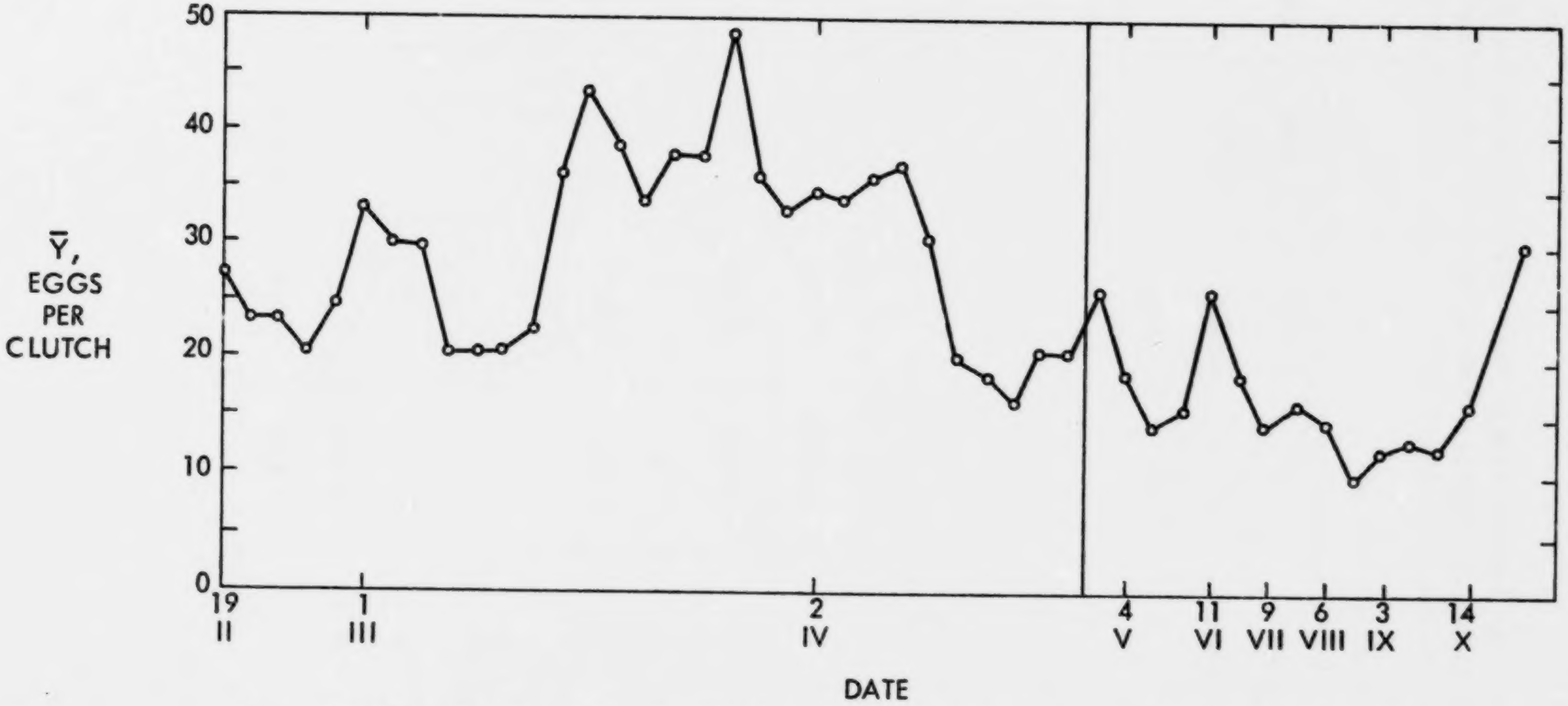

Figure 13. Mean Number of Eggs Per Clutch on Each Collecting Date. The Vertical Line Between 18 April and 24 April Shows a Change in Scale from 2-Day to Approximately 14-Day Intervals.

Table 39. ANOVA TABLE COMPARING THE MEAN RATIOS OF MALES TO FEMALES DURING THE VARIOUS SEASONS.

Source of variation	df	SS	MS	F_s
Among seasons	3	8.2784	2.7594	28.8037*
Within seasons	42	4.0268	0.0958	
Total	45	12.3052		

*P < 0.01

Table 40. *A POSTERIORI* COMPARISON OF THE SEASONAL MEANS OF THE RATIOS OF MALES TO FEMALES USING THE STUDENT-NEWMAN-KEULS TEST

	Summer	Winter	Spring	Fall
$\bar{Y}$	0.9278	0.9938	1.2436	2.7367
n	9	21	14	3
s^2	0.0654	0.0779	0.1489	0.0901
$\bar{Y}$ n				
Su 0.9278 9	--			
W 0.9938 21	0.2515	--		
Sp 1.2436 14	0.3248	0.2178	--	
F 2.7367 3	0.5583*	0.4692*	0.4016*	--

*P< 0.05

Table 41. ANOVA TABLE TESTING THE DIFFERENCES AMONG THE MEAN NUMBERS OF EGGS PER CLUTCH DURING THE VARIOUS SEASONS.

Source of variation	df	SS	MS	F_s
Among seasons	3	1779.9860	593.3286	8.7990*
Within seasons	43	2899.5354	67.4310	
Total	46	4679.5214		

*$P < 0.01$

Table 42. A POSTERIORI COMPARISON OF THE SEASONAL MEAN NUMBERS OF EGGS PER CLUTCH USING THE STUDENT-NEWMAN-KEULS TEST.

	Summer	Fall	Spring	Winter
$\bar{Y}$	15.25	15.33	24.43	30.53
n	9	3	14	21
s^2	23.68	225.33	69.30	67.93
$\bar{Y}$ n				
Su 15.25 9	--			
F 15.33 3	7.5610	--		
Sp 24.43 14	5.8300*	7.2155*	--	
W 30.53 21	5.9875*	8.4225*	3.9130*	--

*$P < 0.05$

per clutch appears to be the most important of the three factors studied in determining the m_x values.

In an attempt to investigate the cause of the variations in the mean number of eggs per clutch, correlation coefficients were calculated between this parameter and each of three environmental factors; i.e. chlorophyll a content in the water, water temperature, and density of adults (Table 43). Significant inverse correlations were found with density and temperature but not with chlorophyll a.

Table 43. CORRELATION COEFFICIENTS FOUND BETWEEN THE MEAN NUMBER OF EGGS PER CLUTCH AND CHLOROPHYLL CONTENT, TEMPERATURE, AND DENSITY OF ADULTS DURING THE STUDY YEAR.

Parameter	n	Correlation Coefficient	P
Chlorophyll	18	-0.3650	nonsignif.
Temperature	47	-0.6003	< 0.01
Adult densities	47	-0.4176	< 0.01

Chlorophyll content has been taken as a quantitative measure of the total food available for grazing in several studies (e.g. Hall, 1964). In a predator-prey (D. clavipes-algae) food relation, an inverse correlation would be expected when the density of the predator increased to the level where the prey were harvested faster than they could reproduce. On the other hand, a positive correlation would be expected when the prey was not limited by the predator, but rather the reproductive intensity of the predator was determined by the amount of prey available. Although an insignificant correlation was found between the mean number of eggs per clutch and chlorophyll content during the full study period, analysis of data during the early spring season (19 February to 31 March) showed a highly significant positive correlation, while a similar analysis for the data from the remainder of the year revealed a significant inverse correlation. Apparently, quantity of food was not a limiting factor during the early spring season, whereas it may have been during the remainder of the year.

Food quality (e.g. species of algae) present would be important in determining the value of chlorophyll as an indicator of available food. In the spring of the year filamentous Spirogyra and colonial Volvox were the dominant genera numerically, while in the summer Ankistrodesmus and Scenedesmus were the dominant algal genera. If the filamentous and colonial algae were of such size that the filter-feeding diaptomids could not ingest them, they would be of no food value to these animals; even though the measured quantity of chlorophyll might have been high.

A second problem relating to food was suggested by the laboratory study on heterogeneity of distribution where it appeared that materials such as detritus and protists might be sources of food. Since the actual food source of D. clavipes is not known, placing too great a value on chlorophyll content alone is tenuous.

Several investigators, including Comita and Anderson (1959), Corkett and McLaren (1969), and Chapman (1969), have suggested that the mean number of eggs per clutch in copepods is correlated to the size of the female. They further state that, since the size a female attains is inversely correlated to the temperature at which she develops, temperature is an important indirect factor in determining the mean number of eggs per clutch. In the current study a significant inverse correlation between temperature and mean number of eggs per clutch was also found.

Figure 14 shows the mean temperatures and the ranges on the various dates of this study. At the onset of the study, the temperature was below the lower limit for successful reproduction as determined in the laboratory (Chapter 3). The temperature steadily increased, however, until 18 August when the yearly maximum of approximately 27°C was reached. After this date the temperature steadily decreased until the termination of the study.

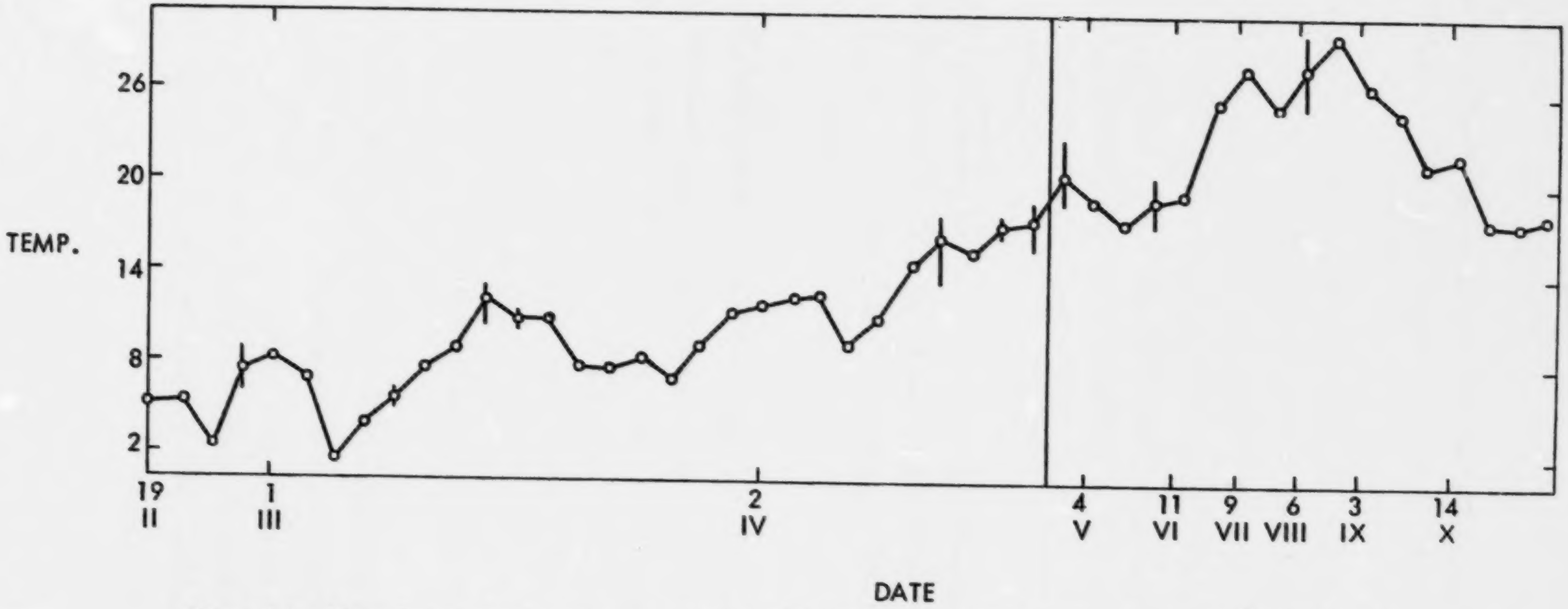

Figure 14. Mean Temperature and Range on Each Collecting Date. The Vertical Line Between 18 April and 24 April Shows a Change in Scale from 2-Day to Approximately 14-Day Intervals.

Although the correlation we observed between temperature and mean number of eggs per clutch agrees with the findings of Chapman (1969), Corkett and McLaren (1969), and Comita and Anderson (1959), the data do not, in themselves, show a cause and effect relationship. In Chapter 3 when we compared the mean number of eggs per clutch at various temperatures, using only data from animals incubated at the same temperature at which they developed, no significant difference was found among the means for the various temperatures, except at 31°C where the mean number of eggs per clutch was lower. These findings suggest that other factors may be more important than temperature in controlling the number of eggs per clutch in the field.

The final factor studied in relation to egg production was adult density (Figure 15). Only the densities in the open water were considered. Concentrations of adults ranged from less than 0.5 to greater than 17 adults per liter during the reproductive year, the higher values were found later in the year. Although the concentration of adults had a 30-fold range during the study period, the total number of adults did not fluctuate to this extent. The greater variation in concentration than in population resulted primarily from the decrease in open water volume due to the encroachment of rooted aquatics.

As with temperature an inverse correlation was found between density and the mean number of eggs per clutch. Figure 16 is a plot of clutch size versus density. It shows a wide range of clutch sizes were observed when densities were below 3 adults per liter, thereby indicating little effect of densities on clutch size at these levels. When the density was greater than 3 adults per liter, however, there are indications that an inverse relationship between the mean number of eggs per clutch and adult density prevailed.

In this study it was found that the major factor affecting the reproductive rate was the mean number of eggs per clutch during the various seasons. Several investigators have correlated the number of eggs per clutch with the size of the female; the size

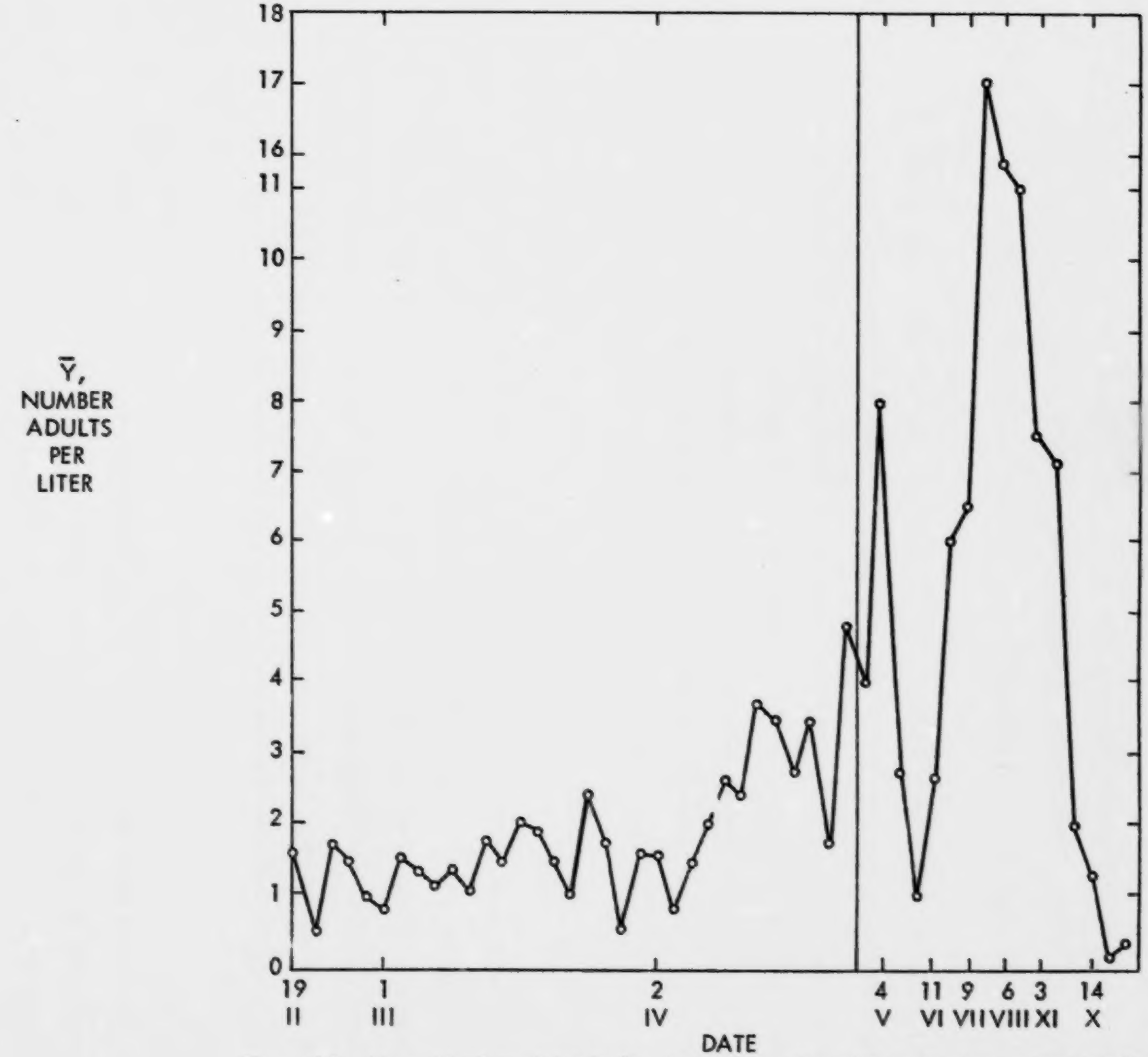

Figure 15. Mean Number of Adults Per Liter in the Open Water Region on Each Collecting Date. The Vertical Line Between 18 April and 24 April Shows a Change in Scale from 2-Day to Approximately 14-Day Intervals.

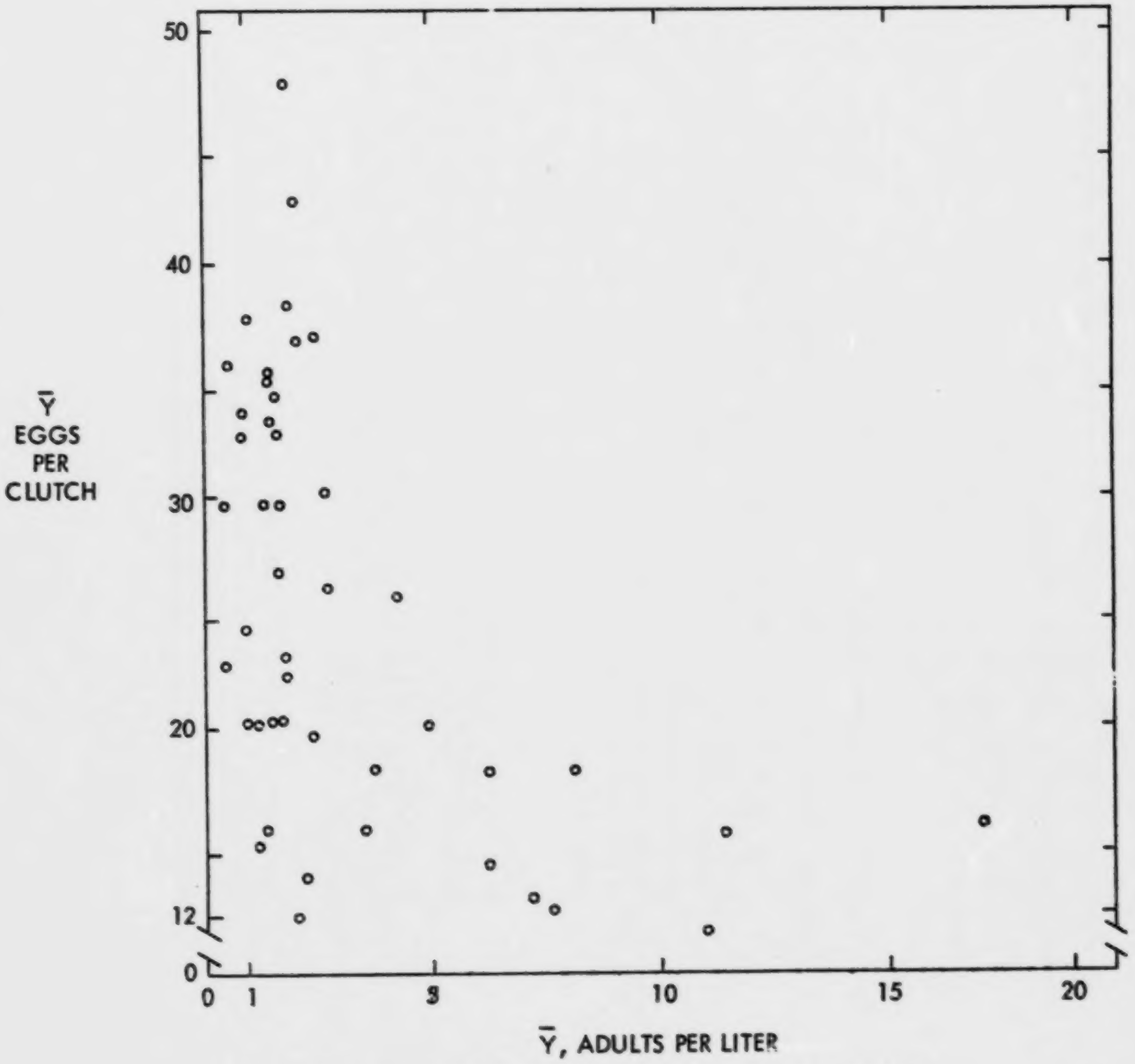

Figure 16. Mean Clutch Size Plotted Against Density of Adults.

being inversely correlated with water temperature during development. Other investigators have found a correlation between the number of eggs per clutch and food. Similar correlations were found between both temperature and food and the mean number of eggs per clutch in the population we studied. Our laboratory data suggest that the significant correlation observed between temperature and clutch size does not necessarily indicate a cause and effect relationship. Interpretation of these data suggest, therefore, that the density of adults may be the regulating mechanism for determining the number of eggs per clutch produced by the females. Selection for a self-regulating mechanism would be expected in a species whose environment is limited and whose individual members are unable to migrate.

SUMMARY

A study dealing with certain aspects of the population dynamics of a field population of Diaptomus clavipes has been carried out. The following results were obtained:

1. When samples were collected every two days from a pond population from 19 February to 20 April and every 14 days from 24 April to 29 October 1971, the adult population was found to be distributed unevenly in the pond. During the entire period but especially in the summer and fall, greater concentrations of copepods occurred in the open water regions than in areas in which rooted aquatics grew close to the surface.

2. Analysis of data from laboratory experiments revealed that, when concentrations of rooted aquatics were present in culture containers, adult animals were unable to survive.

3. Simulating the physical presence of the weeds with pieces of polyethylene tubing caused a retardation in the developmental rate of the egg to NIII stage.

4. Durations of the various instar stages were determined through data obtained during the intensive (every second day) collection period and also through analysis of laboratory data. The durations obtained in the two different ways show quite good agreement.

5. Life tables were constructed for the first generation, the total year, and the laboratory animals. These tables agree in indicating the highest mortality rates occurred in the egg to NIII and the NVI to CI stages.

6. The laboratory population showed substantially greater survival than the field population.

7. The indications are that a physiological survivorship curve characterized the adults of both the field and the laboratory population.

8. Reproduction in the population was analyzed by determining the specific birth rates in the various seasons. Analysis of three factors--male to female ratio, percent of females carrying eggs, and the mean number of eggs per clutch--suggested that the mean number of eggs per clutch was the most important factor in determining the specific birth rates.

9. Chlorophyll a (food), water temperature, and adult densities were studied to evaluate their effects on clutch size. Although significant correlations between the mean number of eggs per clutch and both temperature and adult densities existed, analysis of field and laboratory data suggested that density may be the regulating factor in controlling clutch size and hence reproduction, as long as the temperature is within the range necessary for reproduction in this population.

CHAPTER 5

THE CULTURING OF A CYCLOPOID

In general, freshwater cyclopoid copepods are easier to maintain and culture under laboratory conditions than are calanoids. *Cyclops vernalis* Fischer, the species used in our work, has been cultured previously and used in experimental studies (e.g. Coker, 1933, 1934b, 1934c; Aycock, 1942). Thus, there was no need to develop culturing methods in order to make this form available for use in bioassays or other experimental work.

However, to use a species for such studies, it is very desirable to have some understanding of the relations between culturing conditions and success in order to assure that the methods used are dependable and easily reproducible. This guarantees that experimental animals can always be obtained in the quantities and at the times needed. Also, if the relations between culturing conditions and culturing success are understood, care can be taken so that, when the influence of a certain environmental factor is being studied, the influences of other factors are minimized or controlled. Thus, for example, size of container can be kept large enough so that it does not unduly affect the results obtained in an experiment studying the effects of varying temperature.

The work reported in this chapter had as its objective the development of a dependable, reproducible method for culturing a cyclopoid, specifically *Cyclops vernalis*. Also, this work was intended to clarify the relation between culturing success and certain of the factors influencing it. As an aid in the use of these animals for bioassays, a short section is included on their maintenance and culture in a continuous flow system.

METHODS AND MATERIALS

The methods used in the experiments reported in this chapter were similiar, in most respects, to the methods used in the more extensive study on the effects of temperature on reproduction for C. vernalis reported in the next chapter (Chapter 6). Thus, this section will be kept brief and the Methods and Materials section in that chapter may be referred to if more details are desired.

Our stock cultures of C. vernalis were begun with animals from a dense population of this species found in a freshwater aquarium in the Zoology Building on the University of Oklahoma campus, Norman, Oklahoma. All experiments were conducted at approximately 21°C and under alternating periods of 12-hours light (moderate intensity) and 12-hours dark. The measurements of total dissolved solids (TDS) were made with a Myron L DS Meter (Model 532T 1).

The food used was the trout food-alfalfa mixture whose preparation has been described in Chapter 2. All experimental cultures were fed a predetermined volume of this misture at 2-day intervals. The population in each experimental container was censused every 7 days, and the water in each container was changed at the same time. Unless specified otherwise, pond water filtered through a double layer of #12 bolting silk was used as the culturing medium.

The counting method entailed reducing the volume of water in each experimental container to about 10 ml. The excess water was drawn out of each container by the use of a piece of glass tubing covered at one end by a double layer of #25 bolting cloth and containing a suction bulb at the other end. After the animals had been concentrated in this manner, small volumes of the remaining 10 ml were separately pipetted into a small Petri dish, and the animals present in each volume counted with the aid of a binocular dissecting microscope. The numbers of adults, gravid (egg carrying) females, and young (copepodites plus nauplii) were recorded for each experimental culture at each census time.

A DEPENDABLE, REPRODUCIBLE CULTURING METHOD

The conditions developed for culturing a diaptomid (see Chapter 2) were used as the starting point for culturing Cyclops vernalis. Healthy self-sustaining cultures of the cyclopoid were easily obtained under these conditions. However, in order to gain a greater understanding of the factors affecting culturing success for this species, several sets of experiments considering the effects of varying certain culture conditions were conducted. The results of these experiments are presented in this section.

Culture Volume

The effects of variations in culture volume on culturing success were studied by comparing the size and density of the populations that developed in four different volumes. The culture containers used were 2-inch diameter Petri dishes, 100-ml beakers, 4-inch diameter finger bowls, and 1-liter beakers. These containers were filled with 20 ml, 80 ml, 250 ml, and 1000 ml of water, respectively. Three replicates of each culture volume were used. The volume of food added was adjusted for the different containers so that the rate of addition for each container was 1 ml of food per liter of culture water every second day. Each culture was initiated with 8 adults, 4 males and 4 gravid females.

The average counts obtained during this experiment are presented in Table 44. It will be noted that the greatest numbers of individuals developed in the largest culture volume. This pattern was especially evident with regard to the numbers of adults.

An opposite pattern is noted, however, if one compares the densities rather than the total numbers that developed in each culture volume (Table 45). Here the smallest volume tends to have the greatest density and again the pattern is clearly exhibited by the adults.

Table 44. A COMPARISON OF THE MEAN NUMBERS OF CYCLOPS VERNALIS THAT WERE PRESENT IN FOUR CULTURE VOLUMES ON A SERIES OF SAMPLING TIMES. EACH VALUE IS THE MEAN OF THREE REPLICATES. THE STANDARD ERRORS OF THE MEANS ARE INCLUDED IN PARENTHESES. (A = ADULTS, G = GRAVID FEMALES, Y = IMMATURE COPEPODITES PLUS NAUPLII, T = TOTAL NUMBERS.)

	Culture volume															
	20 ml				80 ml				250 ml				1000 ml			
Week	A	G	Y	T	A	G	Y	T	A	G	Y	T	A	G	Y	T
0	8.0	4.0	0.0	8.0	8.0	4.0	0.0	8.0	8.0	4.0	0.0	8.0	8.0	4.0	0.0	8.0
1	4.7 (0.3)	0.7 (0.7)	6.0 (3.8)	10.7 (3.5)	5.3 (0.7)	1.0 (1.0)	19.3 (9.4)	24.7 (9.5)	7.3 (0.3)	3.3 (0.7)	169.7 (19.1)	177.0 (19.1)	4.0 (1.0)	0.7 (0.3)	29.0 (2.1)	33.0 (2.1)
2	4.7 (0.9)	0.0 (-)	47.0 (14.5)	51.7 (14.7)	13.0 (6.7)	1.7 (0.9)	106.3 (31.9)	119.3 (31.9)	17.7 (5.8)	0.7 (0.3)	183.3 (15.1)	201.0 (20.9)	13.3 (0.7)	1.0 (1.0)	56.3 (22.6)	69.7 (23.2)
3	9.0 (3.0)	0.3 (0.3)	45.3 (2.6)	54.3 (3.8)	19.7 (8.7)	1.3 (0.7)	40.3 (7.4)	60.0 (16.0)	13.0 (3.0)	0.7 (0.7)	103.7 (35.6)	116.7 (38.6)	37.3 (10.2)	5.3 (2.3)	202.0 (79.3)	239.3 (88.8)
4	4.7 (1.2)	0.0 (-)	30.3 (10.5)	35.0 (11.7)	16.3 (5.5)	1.0 (0.6)	49.0 (13.3)	65.3 (18.5)	25.7 (2.4)	1.0 (0.6)	43.0 (22.2)	68.7 (24.3)	63.3 (25.9)	3.7 (1.2)	95.0 (39.2)	158.3 (16.6)
5	7.7 (1.2)	0.0 (-)	21.3 (2.0)	29.0 (2.5)	19.7 (2.2)	2.0 (0.6)	114.3 (28.8)	134.0 (30.7)	22.0 (4.0)	3.7 (1.3)	145.0 (93.0)	167.0 (94.5)	50.3 (10.3)	4.7 (2.2)	149.7 (61.5)	200.0 (69.4)
6	6.0 (1.5)	0.0 (-)	101.7 (45.9)	107.7 (47.4)	20.0 (7.5)	1.3 (0.3)	132.0 (37.9)	152.0 (44.4)	21.7 (1.5)	3.0 (2.1)	334.3 (149.3)	356.0 (150.7)	36.0 (10.1)	8.0 (2.5)	37.7 (9.3)	73.7 (19.0)
7	7.7 (1.2)	0.0 (-)	52.0 (24.8)	59.7 (26.0)	20.3 (7.3)	0.7 (0.3)	142.0 (20.2)	162.3 (14.6)	26.7 (7.7)	2.7 (1.7)	206.0 (53.4)	232.7 (60.1)	41.3 (12.0)	9.0 (4.6)	306.0 (108.9)	347.3 (117.1)
8	9.0 (0.6)	1.0 (0.6)	57.3 (6.1)	66.3 (5.8)	19.3 (3.3)	0.3 (0.3)	108.7 (7.6)	128.0 (7.8)	37.3 (20.9)	1.3 (0.3)	61.7 (18.3)	99.0 (26.1)	55.0 (9.0)	8.0 (2.3)	183.3 (68.4)	238.3 (77.2)
9	9.0 (1.5)	1.0 (0.6)	64.0 (22.1)	73.0 (21.3)	19.7 (2.3)	0.7 (0.7)	110.7 (44.4)	130.3 (46.5)	33.3 (10.5)	0.7 (0.7)	70.7 (34.3)	104.0 (44.5)	67.0 (12.7)	7.7 (1.8)	260.7 (54.1)	327.7 (48.3)

Table 45. A COMPARISON OF THE MEAN DENSITIES OF *CYCLOPS VERNALIS* THAT WERE PRESENT IN FOUR CULTURE VOLUMES ON A SERIES OF SAMPLING TIMES. THESE DENSITIES ARE BASED ON THE DATA IN TABLE 44. (A = ADULTS, G = GRAVID FEMALES, Y = IMMATURE COPEPODITES PLUS NAUPLII, T = TOTAL NUMBERS.)

	Culture volume															
	20 ml				80 ml				250 ml				1000 ml			
Week	A	G	Y	T	A	G	Y	T	A	G	Y	T	A	G	Y	T
0	400	200	0	400	100	50	0	100	32	16	0	32	8	4	0	8
1	235	35	300	535	66	12	241	309	29	13	679	708	4	1	29	33
2	235	0	2350	2585	162	21	1329	1491	71	3	733	804	13	1	56	10
3	450	15	2265	2715	246	16	504	750	52	3	415	467	37	5	202	239
4	235	0	1515	1750	204	12	612	816	103	4	172	275	63	4	95	158
5	385	0	1065	1450	246	25	1429	1675	88	15	580	668	50	5	150	200
6	300	0	5085	5385	250	16	1650	1900	87	12	1337	1424	36	8	38	74
7	385	0	2600	2985	254	9	1775	2029	107	11	824	931	41	9	306	347
8	450	50	2865	3315	241	4	1359	1600	149	5	247	396	55	8	183	238
9	450	50	3200	3650	246	9	1384	1629	133	3	283	416	67	8	261	328

This work indicates that culture volume over the range studied in this experiment does not greatly affect culturing success. More animals developed in the larger volumes but the highest densities were noted in the smallest volume. These differences are probably related to food availability in the different volumes. These differences aside, however, it was generally observed that the cultures at all four volumes were very successful. There seems little indication of culturing becoming more difficult at low volumes as was observed for *Diaptomus clavipes*.

Food Concentration

Two separate tests were conducted to determine the effects on culturing success of varying food concentration. In the first test, 9 separate cultures were initiated in 1-liter beakers by adding 8 adults, 4 males and 4 gravid females, to each beaker. These cultures were divided into three sets of three replicates each. One set of replicates received 0.1 ml of food solution every second day, one set 0.5 ml, and one set 1.0 ml.

The results from this experiment are presented in Table 46. It is immediately obvious that vigorous cultures developed at all three feeding rates. There is some indication that the population density increased with the amount of food added. This is quite evident for the adults but not so clear for the total numbers,where the densities in the cultures receiving 0.5 ml seem to have been at least as great as those in the cultures receiving 1.0 ml.

A second test was run in much the same way as the one just described. However, 4-inch finger bowls each containing 250 ml of culture water were used instead of 1-liter beakers, and the food volumes added were somewhat different. One set of three replicates recieved food at the rate of 0.4 ml per liter of culture water ever second day, in other words 0.1 ml were added to the 250-ml cultures each time. The second set received 1.0 ml per liter every second day, and the third set received 2.0 ml per liter every second day.

Table 46. A COMPARISON OF THE MEAN NUMBERS OF CYCLOPS VERNALIS FOUND UNDER THREE DIFFERENT RATES OF FOOD ADDITIONS ON A SERIES OF SAMPLING DATES. EACH VALUE IS THE MEAN OF THREE REPLICATES. THE STANDARD ERRORS OF THE MEANS ARE INCLUDED IN PARENTHESES. (A = ADULTS, G = GRAVID FEMALES, Y = IMMATURES PLUS NAUPLII, T = TOTAL NUMBERS.)

	Rate of food addition every second day											
	0.1 ml/liter				0.5 ml/litter				1.0 ml/liter			
Week	A	G	Y	T	A	G	Y	T	A	G	Y	T
0	8.0	4.0	0.0	8.0	8.0	4.0	0.0	8.0	8.0	4.0	0.0	8.0
1	8.0 (1.0)	3.0 (0.6)	118.7 (105.7)	126.7 (106.2)	7.0 (1.0)	2.0 (0.0)	27.0 (5.1)	34.0 (4.2)	4.0 (1.0)	0.7 (0.3)	29.0 (2.1)	33.0 (2.1)
2	11.0 (1.7)	1.0 (1.0)	106.7 (38.3)	117.7 (40.0)	11.7 (1.5)	2.7 (0.9)	82.0 (23.3)	93.7 (22.0)	13.3 (0.7)	1.0 (1.0)	56.3 (22.6)	69.7 (23.2)
3	17.0 (5.6)	0.7 (0.3)	92.7 (37.7)	109.7 (41.9)	27.7 (5.9)	2.7 (0.9)	87.0 (3.2)	114.7 (8.7)	37.3 (10.2)	5.3 (2.3)	202.0 (79.3)	239.3 (88.8)
4	27.3 (9.6)	0.0 (-)	78.3 (34.5)	105.7 (44.1)	38.0 (0.0)	7.7 (2.4)	151.7 (84.4)	189.7 (84.4)	63.3 (25.9)	3.7 (1.2)	95.0 (39.2)	158.3 (16.6)
5	22.3 4.1	0.3 (0.3)	40.0 (9.5)	62.3 (11.1)	37.3 (5.2)	2.0 (1.0)	240.7 (148.5)	278.0 (150.3)	50.3 (10.3)	4.7 (2.2)	149.7 (61.5)	200.0 (69.4)
6	13.7 (3.0)	1.0 (0.6)	48.7 (31.4)	62.3 (32.5)	29.7 (7.9)	4.3 (1.7)	151.0 (97.6)	180.7 (105.3)	36.0 (10.1)	8.0 (2.5)	37.7 (9.3)	73.7 (19.0)
7	18.0 (4.4)	1.0 (0.6)	79.7 (34.2)	97.7 (38.3)	33.3 (10.9)	2.0 (1.5)	320.3 (71.2)	353.7 (70.3)	41.3 (12.0)	9.0 (4.6)	306.0 (108.9)	347.3 (117.1)
8	18.7 (4.9)	1.3 (1.3)	86.0 (12.9)	104.7 (16.5)	43.3 (2.4)	1.3 (0.7)	225.0 (74.5)	268.3 (72.3)	55.0 (9.0)	8.0 (2.3)	183.3 (68.4)	238.3 (77.2)
9	18.0 (4.0)	1.0 (1.0)	313.5 (47.5)	331.5 (51.5)	45.5 (2.5)	3.0 (1.0)	208.5 (3.5)	254.0 (1.0)	67.0 (12.7)	7.7 (1.8)	260.7 (54.1)	327.7 (48.3)
10	28.5 (0.5)	2.0 (0.0)	308.5 (19.5)	337.0 (20.0)	48.0 (8.0)	4.0 (2.0)	477.0 (118.0)	525.0 (110.0)	53.3 (5.5)	16.0 (4.9)	539.3 (94.9)	592.7 (94.3)

The results from this second experiment are presented in Table 47. They agree with the preceding work in showing successful cultures at all three feeding levels. These results also show increases in density associated with increasing amounts of food added. In this experiment the increase in density with food seemed to hold for the numbers of total individuals as well as the numbers of adults.

This work indicates that, over the range of food concentrations studied, successful cultures are produced irregardless of feeding level. There are no indications of the culturing failures noted for Diaptomus clavipes at rates of food addition above 0.1 or 0.2 ml every second or third day. In fact, the indications are that the culture densities for C. vernalis increase with increasing food at least up to the highest level attempted, 2.0 ml per liter every second day.

Water Quality

An attempt was made to learn something concerning the effects of the chemical composition of the water used for culturing on culturing success. As a simple first step in this type of study, the effects of culturing in waters from four different sources were monitored. As an overall indication of the quality of these waters, the concentrations of total dissolved solids (TDS) were measured. Obviously, TDS is only one chemical property of many that may vary among various waters and thus affect their suitability for raising copepods. However, it was considered as good an indicator as any of general chemical conditions and thus was considered an appropriate property to measure in an initial study.

Populations were initiated in 100 ml beakers, each containing approximately 60 ml of pond water, by adding 6 adults, 3 of each sex. Waters of four types were tested. The sources of these four waters were: 1) Noble water--from wells on the floodplain of the South Canadian River near the town of Noble, Cleveland County, Oklahoma (TDS approximately 1,000 ppm); 2) tap water--the water from the taps

Table 47. A COMPARISON OF THE MEAN NUMBERS OF CYCLOPS VERNALIS FOUND UNDER THREE DIFFERENT RATES OF FOOD ADDITIONS ON A SERIES OF SAMPLING DATES. EACH VALUE IS THE MEAN OF THREE REPLICATES. THE STANDARD ERRORS OF THE MEANS ARE INCLUDED IN PARENTHESES. (A = ADULTS, G = GRAVID FEMALES, Y = IMMATURE COPEPODITES PLUS NAUPLII, T = TOTAL NUMBERS.)

	Rate of food addition every second day											
	0.4 ml/liter				1.0 ml/liter				2.0 ml/liter			
Week	A	G	Y	T	A	G	Y	T	A	G	Y	T
0	8.0	4.0	0.0	8.0	8.0	4.0	0.0	8.0	8.0	4.0	0.0	8.0
1	6.7 (0.3)	2.7 (0.9)	147.7 (14.2)	154.3 (14.3)	7.3 (0.3)	3.3 (0.7)	169.7 (19.1)	177.0 (19.1)	5.7 (1.2)	1.3 (0.3)	82.0 (24.6)	87.7 (24.8)
2	13.7 (1.3)	2.0 (1.0)	196.3 (50.2)	210.0 (51.4)	17.7 5.8	0.7 (0.3)	183.3 (15.1)	201.0 (20.9)	17.0 (7.6)	2.3 (1.3)	198.0 (83.5)	215.0 (91.1)
3	11.0 (0.0)	0.3 (0.3)	88.0 (9.7)	99.0 (9.7)	13.0 (3.0)	0.7 (0.7)	103.7 (35.6)	116.7 (38.6)	16.7 (4.9)	0.0 (-)	93.7 (25.2)	107.3 (26.4)
4	21.3 (0.9)	1.3 (0.9)	55.0 (2.9)	76.3 (3.3)	25.7 (2.4)	1.0 (0.6)	43.0 (22.2)	68.7 (24.3)	24.7 (2.4)	4.0 (1.2)	100.3 (27.3)	125.0 (27.5)
5	17.7 (2.3)	0.7 (0.3)	59.0 (17.8)	76.7 (19.7)	22.0 (4.0)	3.7 (1.3)	145.0 (93.0)	167.0 (94.5)	23.7 (7.2)	5.3 (1.5)	273.0 (97.8)	296.7 (103.0)
6	19.7 (2.0)	3.3 (1.7)	146.0 (54.0)	165.7 (56.1)	21.7 (1.5)	3.0 (2.1)	334.3 (149.3)	356.0 (150.7)	38.0 (6.4)	3.7 (2.7)	191.0 (30.1)	229.0 (36.3)
7	19.7 (2.9)	3.3 (1.2)	121.3 (52.1)	141.0 (49.2)	26.7 (7.7)	2.7 (1.7)	206.0 (53.4)	232.7 (60.1)	41.7 (8.8)	2.7 (1.5)	217.7 (89.6)	259.3 (80.8)
8	23.7 (0.3)	3.7 (1.2)	149.7 (45.8)	173.3 (45.9)	37.3 (20.9)	1.3 (0.3)	61.7 (18.3)	99.0 (26.1)	54.3 (9.7)	3.7 (0.9)	117.7 (54.6)	172.0 (56.8)
9	22.3 (1.2)	1.0 (0.6)	106.3 (26.5)	128.7 (26.0)	33.3 (10.5)	0.7 (0.7)	70.7 (34.3)	104.0 (44.5)	44.7 (7.0)	6.3 (4.4)	144.3 (56.5)	189.0 (51.8)
10	23.7 (1.2)	1.3 (1.3)	134.3 (41.4)	158.0 (42.5)	35.3 (13.5)	3.0 (1.5)	108.7 (37.0)	144.0 (34.9)	52.3 (8.9)	7.7 (2.0)	168.0 (50.9)	220.3 (53.0)
11	18.7 (1.9)	1.3 (0.9)	68.7 (38.8)	87.3 (40.3)	38.0 (12.1)	5.0 (1.5)	112.0 (57.6)	150.0 (61.5)	49.0 (8.7)	6.3 (2.4)	147.7 (51.4)	196.7 (60.0)

at the University of Oklahoma (TDS approximately 500 ppm), this water orginated from wells on the University campus; 3) pond water--from a stock pond near Noble (TDS approximately 110 ppm); and 4) distilled water--low quality distilled water produced by a general purpose still at the University (TDS approximately 25 ppm). Also two mixtures were made up with resultant TDS values similar to those for the pond water. One mixture was composed of 1-part Noble water to every 9 parts of distilled water, resulting in a TDS of approximately 125 ppm. The second mixture was composed of 1 part tap water for every 4 parts distilled water; this had an approximate TDS value of 110 ppm.

Five replicate cultures were monitored for each type of water making a total of 30 cultures. The results from this experiment (expressed as means) are presented in Table 48. Successful cultures developed in all the types of water tested. However, there were differences among the population densities that developed in the different water types.

To look at these differences more closely, the numbers for total individuals for the six types of water have been ranked from highest (rank 1) to lowest (rank 6) separately for each week (Table 49). The same procedure has been followed for the numbers of adults (Table 50). It may be noted in both tables that the pond water had the highest or almost the highest numbers all the time. The other water types were roughly similar in the results obtained. Certainly there is no evidence that the two mixtures which had TDS values similar to those for the pond water produced more animals than the waters with higher or lower TDS readings.

From this work it is concluded that the pond water was somewhat better than the other waters tested for culturing. Perhaps this superiority was due to nothing more than the small particles or organic matter that were not removed from the water by the #12 silk used for filtering, providing an extra source of food. Whatever caused the pond water to be somewhat superior, it certainly was not related to TDS value as the waters

Table 48. A COMPARISON OF THE MEAN NUMBERS OF CYCLOPS VERNALIS PRESENT ON A SERIES OF SAMPLING DATES IN SIX DIFFERENT TYPES OF CULTURING WATER. EACH VALUE IS THE MEAN OF FIVE REPLICATES. THE STANDARD ERRORS OF THE MEANS ARE INCLUDED IN PARENTHESES. (A = ADULTS, G = GRAVID FEMALES, Y = IMMATURE COPEPODITES PLUS NAUPLII, T = TOTAL NUMBERS.)

Week	Type of water											
	Noble (TDS app. 1000)				Pond (TDS app. 110)				Tap (TDS app. 500)			
	A	G	Y	T	A	G	Y	T	A	G	Y	T
0	6.0	0.0	0.0	6.0	6.0	0.0	0.0	6.0	6.0	0.0	0.0	6.0
1	6.6 (0.4)	0.6 (0.4)	17.6 (11.3)	24.2 (11.1)	9.0 (1.7)	1.8 (0.5)	155.8 (28.3)	164.8 (29.5)	7.2 (0.4)	0.6 (0.4)	98.8 (26.7)	106.0 (27.0)
2	4.0 (0.3)	0.6 (0.2)	74.4 (13.3)	78.4 (13.2)	10.6 (1.7)	2.0 (0.9)	142.4 (24.1)	153.0 (24.5)	8.0 (1.5)	0.6 (0.4)	115.6 (26.2)	123.6 (26.0)
3	12.0 (2.5)	0.6 (0.2)	46.0 (6.2)	58.0 (8.2)	11.2 (1.2)	0.6 (0.2)	151.0 (28.3)	162.2 (29.4)	6.6 (1.0)	0.4 (0.2)	41.4 (7.6)	48.0 (8.0)
4	10.2 (1.3)	0.2 (0.2)	50.6 (11.0)	60.8 (10.3)	13.2 (0.6)	0.4 (0.4)	135.8 (28.7)	149.0 (29.0)	8.8 (1.2)	0.2 (0.2)	37.4 (9.2)	46.2 (9.8)
5	17.0 (2.2)	0.0 (-)	39.4 (8.7)	56.4 (8.7)	17.8 (1.6)	0.6 (0.4)	100.6 (12.9)	118.4 (12.8)	12.4 (1.8)	0.2 (0.2)	35.2 (14.4)	47.6 (13.8)
6.	21.4 (0.8)	0.4 (0.2)	42.6 (6.9)	64.0 (7.2)	24.6 (2.4)	0.2 (0.2)	103.6 (20.1)	128.2 (19.1)	15.2 (1.9)	0.2 (0.2)	28.8 (8.0)	44.0 (8.1)
7	22.2 (1.0)	1.4 (0.5)	34.2 (4.0)	56.4 (3.8)	27.7 (4.2)	0.5 (0.3)	67.2 (13.6)	95.0 (16.1)	12.8 (1.9)	0.0 (-)	38.6 (16.7)	51.4 (18.1)
8	19.0 (2.2)	0.8 (0.4)	22.0 (4.8)	41.0 (5.6)	26.2 (2.2)	1.0 (0.6)	44.2 (11.2)	70.5 (11.1)	11.8 (2.2)	0.6 (0.6)	20.2 (8.8)	32.0 (11.0)

Table 48 (Cont.)

Week	Type of water											
	Distilled (TDS app. 25)				1/10 Noble, 9/10 Dist. (TDS app. 125)				1/5 Tap, 4/5 Dist. (TDS app. 110)			
	A	G	Y	T	A	G	Y	T	A	G	Y	T
0	6.0	0.0	0.0	6.0	6.0	0.0	0.0	6.0	6.0	0.0	0.0	6.0
1	7.4 (1.0)	0.6 (0.4)	146.6 (14.3)	154.0 (15.1)	6.0 (0.6)	0.6 (0.6)	99.0 (34.7)	105.0 (34.8)	4.2 (0.6)	0.2 (0.2)	113.4 (29.8)	117.6 (29.5)
2	6.8 (1.4)	1.2 (0.6)	112.4 (17.9)	119.2 (19.1)	6.6 (1.2)	1.0 (0.3)	48.8 (12.0)	55.4 (12.6)	3.4 (0.5)	0.8 (0.4)	98.6 (25.8)	102.0 (25.6)
3	6.6 (1.6)	0.6 (0.2)	101.2 (25.3)	107.8 (26.0)	9.6 (2.1)	0.8 (0.5)	77.8 (24.7)	87.4 (24.9)	3.0 (1.0)	0.0 (-)	87.4 (17.9)	90.4 (17.5)
4	6.8 (0.9)	1.0 (0.5)	63.4 (12.8)	70.2 (12.8)	9.4 (2.3)	0.6 (0.6)	56.8 (19.3)	66.2 (17.5)	5.6 (1.7)	0.4 (0.4)	34.6 (7.6)	40.2 (8.2)
5	9.0 (1.2)	1.2 (0.5)	100.8 (25.8)	109.8 (25.7)	8.8 (3.0)	0.2 (0.2)	29.2 (13.8)	38.0 (12.5)	9.2 (3.0)	0.2 (0.2)	31.4 (17.1)	40.6 (17.3)
6	13.6 (1.3)	1.0 (0.6)	111.4 (32.6)	125.0 (32.1)	11.4 (1.4)	0.2 (0.2)	33.2 (11.1)	44.6 (12.4)	10.2 (3.1)	0.0 (-)	26.8 (10.2)	37.0 (10.5)
7	9.6 (1.3)	1.0 (0.4)	88.0 (14.8)	97.6 (16.0)	11.2 (0.5)	1.0 (0.4)	82.8 (30.7)	94.0 (30.9)	11.4 (4.0)	0.2 (0.2)	38.4 (13.7)	49.8 (17.0)
8	10.8 (1.4)	1.0 (0.5)	41.8 (13.0)	52.6 (14.2)	10.4 (1.2)	2.2 (0.7)	77.6 (23.9)	88.0 (24.4)	7.2 (2.3)	0.8 (0.4)	17.6 (5.9)	24.8 (7.0)

Table 49. A COMPARISON OF THE RANKS OF TOTAL NUMBERS OF *CYCLOPS VERNALIS* (AS SHOWN IN TABLE 48) KEPT IN SIX DIFFERENT TYPES OF WATER. THE SIX WATERS WERE RANKED FROM HIGHEST TOTAL NUMBER OF COPEPODS (RANK 1) TO LOWEST (RANK 6) SEPARATELY FOR EACH WEEK OF SAMPLING

Week	Type of water					
	Noble	Pond	Tap	Distilled	1/10 Noble 9/10 Dist.	1/5 Tap 4/5 Dist.
1	6	1	5	2	4	3
2	5	1	2	3	6	4
3	5	1	6	2	4	3
4	4	1	6	2	3	5
5	3	1	4	2	6	5
6	3	1	5	2	4	6
7	4	2	5	1	3	6
8	4	2	5	3	1	6
Total of ranks	34	10	38	17	31	38
App. TDS	1000	110	500	25	125	110

Table 50. A COMPARISON OF THE RANKS OF THE NUMBERS OF ADULT *CYCLOPS VERNALIS* (AS SHOWN IN TABLE 48) KEPT IN SIX DIFFERENT TYPES OF WATER. THE SIX WATERS WERE RANKED FROM HIGHEST NUMBER OF ADULTS (RANK 1) TO LOWEST (RANK 6) SEPARATELY FOR EACH WEEK OF SAMPLING

	Type of water					
Week	Noble	Pond	Tap	Distilled	1/10 Noble 9/10 Dist.	1/5 Tap 4/5 Dist.
1	4.5	1.5	1.5	3	4.5	6
2	5	1	2	3.5	3.5	6
3	1	2	4.5	4.5	3	6
4	2	1	3.5	5	3.5	6
5	2	1	3	5	5	5
6	2	1	3	4	5	6
7	2	1	3	6	4.5	4.5
8	2	1	3	4	5	6
Total of ranks	20.5	9.5	23.5	35	34	45.5
App. TDS	1000	110	500	25	125	110

with TDS readings close to those of the pond water showed no better results than the Noble water with a TDS of 1000 or the so-called distilled water with a TDS of 25.

Cannibalism

Cannibalism, especially by the mother on her newly hatched offspring, was observed several times during our research and has been reported in cyclopoids by several other authors. Fryer (1957) reported *C. vernalis* as a carnivorous copepod, having found cyclopoid remains in the guts of individuals of this species. Khan (1965) and Smyly (1970) both noted high mortality of nauplii when left in the presence of adult *Acanthocyclops viridis*, but neither reported the magnitude of this mortality.

To determine the extent of the mortality of newly hatched *C. vernalis* nauplii when in the presence of their mother, the following experiment was carried out. Twenty gravid females were placed in separate 5-cm Petri dishes, each containing 10 ml of filtered pond water and maintained at 21°C. Within 2 hours after the clutch carried by each female had hatched, the nauplii were counted. Also at that time, the females were removed from ten of the dishes. The nauplii in each dish were counted again 24 and 48 hours after the first count (Appendix F-1).

At both 24 and 48 hours after the first count, the mean percentage of nauplii surviving in dishes with females was approximately one-half that of the nauplii which were in dishes from which the females had been removed (Table 51). Student's t-test comparing the percentages with and without the mother present at both 24 and 48 hours showed differences between the two groups to be highly significant at both times ($P < 0.001$). Further, using the same test it was found that for both the dishes with adult copepods and those without, the mean percentage of nauplii surviving for 48 hours did not differ significantly ($P > 0.05$) from the mean percentage surviving for 24 hours.

Table 51. PERCENTAGE OF NAUPLII OF *CYCLOPS VERNALIS* SURVIVING 24 AND 48 HOURS AFTER HATCHING WITH AND WITHOUT THE PRESENCE OF THE MOTHER.

	Percentage of nauplii surviving	
	24 hours	48 hours
	Mother Present	
Mean	53.9	41.8
$SE_{\bar{x}}$	±4.95	±5.13
min.	30.0	12.0
max.	83.0	63.0
	Mother Removed	
Mean	98.4	96.2
$SE_{\bar{x}}$	±0.56	±1.18
min.	96.0	88.0
max.	100.0	100.0

These results indicate that a high mortality among newly hatched C. vernalis nauplii can result when they are in the presence of an adult. Smyly (1970) stated that such cannibalism depends directly on the frequency of encounter of the mother with the nauplii. In our results mortality of approximately 50% occurred in the first 24-hour period, whereas only about 20% mortality of the remaining animals occurred during the second 24-hour period. Possibly an increase in the size of the nauplii or an increase in their mobility may have caused this decrease in mortality during the second day after hatching.

Temperature

A study specifically designed to investigate the relation between temperature and certain reproductive attributes for C. vernalis is reported in the next chapter (Chapter 6). Because that study provided a good deal of information on the influence of temperature on populations of C. vernalis, no experiments in which temperature was varied were conducted during the research on culturing reported in the present chapter.

When the results from Chapter 6 are examined, it is clear that the animals reproduced quite well anywhere within a range from about 14° to 31°C. The rate of reproduction was retarded somewhat in the lower part of this range, however, and the number of eggs produced by a female during her life was reduced at the upper temperature. With these results in mind it seems prudent to suggest a temperature between 20° and 25° C for culturing of this species.

CONTINUOUS FLOW CULTURE

It has been noted that many organisms are more sensitive to a toxic material when they are exposed to a certain concentration of it in a continuous flow system than when they are exposed to the same concentration in a standing water system. Thus, to assure that individuals of C. vernalis cultured by the methods employed in our work could be used in continuous flow bioassays, an attempt has been made to culture them in a continuous flow system. This section describes the system used and reports on the attempts to culture C. vernalis in this system.

The Continuous Flow System

Basically the continuous flow system consisted of a reservoir of culture medium (filtered pond water) which was caused to flow through a culture container by the action of an electrolysis pump. The pump consisted of a flask containing dilute H_2SO_4 in which was immersed two platinum wire poles. A transformer connected to the electrodes reduced the voltage from a wall plug to a level of 3 to 6 volts. The O_2 and H_2 produced in the electrolysis flask flowed through glass tubing into the gas space of a large,sealed container which contained 10 liters of culture medium. The water from this reservoir flowed out through a glass tube which was immersed almost to the bottom of the container. This tube terminated just inside the top of a sealed 1-liter culture flask where the fresh medium dripped into the culture. The added water caused some of the medium in the culture to flow out another piece of tubing which was immersed in the culture almost to the bottom as was the tubing in the reservoir container. This outflow tubing was covered with #25 plankton netting. The flow through this system was approximately 2 liters per day.

The Culturing Results

A culture of C. vernalis was initiated in the continuous flow system by adding 6 adult males and 4 gravid females to the culture container. Two milliliters of the fish food-alfalfa mixture were added to this culture every second day. Table 52 shows the number of adults, females, young, and total individuals found in this culture after 4 weeks. It will be noted that the culture increased. Reproduction obviously occurred and some of the young had matured by the time of sampling. Thus, it is concluded that this species can be cultured in a continuous flow system.

Table 52. NUMBERS OF CYCLOPS VERNALIS AFTER 4 WEEKS IN A CONTINUOUS FLOW CULTURING SYSTEM.

Week	Numbers of individuals			
	Males	Females	Immature copepodites	Nauplii
0	6	4	0	0
4	8	22	8	48

Several problems were encountered in the continuous flow experiment, however. Fine mesh bolting cloth (#25) was placed over the end of the culture container outlet tube in an attempt to prevent the escape of young nauplii. Even so, many of the smallest nauplii were noted to pass through this netting. To prevent this escape the layer of netting over the end of the tube was doubled. This seemed to prevent the escape of most of the nauplii but led to other problems.

The double-thickness netting became clogged quite quickly with food particles which greatly impeded the flow through the system. This problem was partially overcome by connecting a small inverted funnel to the end of the outlet tube so that the water from the culture entered the tubing through the wide end of the funnel. This outlet was then covered with a double thickness of #25 netting. The increased area of this outlet decreased the clogging problem while still providing the safeguard of a double thickness of netting to retain the nauplii. Another aid to reduce clogging was provided by filtering the food mixture through #25 bolting silk while it was being produced, rather than through the #20 silk usually used.

Even with the refinements mentioned above, clogging of the netting was some problem. Overall it seems, at least at present, that culturing C. vernalis in a continuous flow system is more difficult and time-consuming than culturing the species in a standing water system. If bioassays concerning the toxic effects of certain materials on adults of this species are to be conducted, it may well be advisable to culture the animals in standing water systems and then transfer them to continuous flow ones for the measurement of toxic effects.

SUMMARY

An investigation has been conducted with the objective of specifying a set of conditions that will allow dependable, reproducible culturing of Cyclops vernalis. Based on this work the following conditions are recommended for culturing:

1. Culture volume--Comparing the size and density of the populations that developed in volumes of 20, 80, 250 and 1000 ml showed that this factor had little effect on the culturing success for C. vernalis, at least in the range from 20 to 1000 ml. Cultures with volumes of about 100 ml should be convenient for many experimental purposes.

2. Food type--Only one type of food was used during this work. This food, a fish food-alfalfa mixture, proved quite satisfactory and is recommended.

3. Food quantity--Successful cultures developed at rates of food addition from 0.1 to 2.0 ml of food mixture per liter of culture medium every second day. The densities that developed in the cultures seemed to be directly related to quantity of food addition, although not on a linear basis. Food additions anywhere in the above range are recommended with the exact amount determined by the density desired.

4. Culture medium--Culturing was attempted with four types of water, i.e. Noble water (well water), tap water, pond water, and low quality distilled water. Successful populations developed in all four water types. However, pond water is suggested for culturing because the densities were higher in it than in the other types. The other three types developed approximately equal densities.

5. Total dissolved solids--Two mixtures, one of Noble water (TDS = 1000 ppm) plus distilled water (TDS = 25 ppm) and one of the tap water (TDS = 500 ppm) plus distilled water, were prepared so that both had a TDS value approaching that of the pond water (TDS = 110 ppm). The population densities

that developed in the mixtures were no greater than those in the waters from which they were prepared. Thus, the total dissolved solids value can not be detected as having an effect on culturing success.

6. Removal of adults--A comparison of the amounts of mortality for newly hatched nauplii with their mothers present and removed showed much higher mortalities with the mothers present, suggesting cannibalism. Thus, to maximize culturing success, egg-carrying females should be placed in separate containers and then removed as soon as their eggs hatch.

7. Temperature--No experiments concerning the effects of varying this factor were conducted in the work reported in this chapter. However, based on the results from the studies in the next chapter, a temperature in the range 20 to 25°C is recommended for culturing.

An experiment was conducted to determine if *C. vernalis* can be cultured in a continuous flow system. The system employed is described in the text, and its flow was driven by an electrolysis pump. Although the experiment only ran for 4 weeks, a population which appeared to be self-sustaining developed in the system. Nauplii escaped with the outflow water in this system unless fine netting was used to cover the outlet. Unfortunately, the netting tended to clog with food particles thus impeding flow. This problem could be corrected, to some extent, by increasing the area of the outflow, but it made continuous flow culturing with the system used less satisfactory than culturing in a standing water system.

CHAPTER 6

THE INFLUENCE OF TEMPERATURE ON THE REPRODUCTION OF *CYCLOPS VERNALIS*

If we are to protect the quality of our aquatic environments, we should possess a thorough understanding of the environmental relations of the common aquatic organisms. The cyclopoid copepod *Cyclops vernalis* Fischer is one of the most abundent and widely distributed planktonic forms in North America, occurring over most of the continent and in a variety of habitats (Yeatman, 1944). Thus, it was deemed important to increase our knowledge concerning this form. Such added knowledge should aid our understanding of the ecological relations, not only of this species, but of planktonic cyclopoid copepods in general.

The development of a dependable culturing method reported in the preceding chapter was the first step in this work. Using these methods experimental studies on environmental relations could be conducted.

A number of studies, including those of Ewers (1936), Andrews (1953), Roen (1955), Elgmork (1959), and Armitage and Tash (1967), have suggested temperature as a very important influence on cyclopoids. We have conducted a study whose primary objective was to investigate the influence of this factor on certain reproductive attributes of *C. vernalis*. The results from this study are presented in this chapter.

METHODS AND MATERIALS

Stock Cultures

Stock cultures of *Cyclops vernalis* were set up in 1-liter beakers at temperatures of 14°, 21°, 26°, and 31°C. All animals used to begin these cultures were collected from a dense population contained in a freshwater aquarium maintained in the Zoology Building on the University of Oklahoma campus, Norman, Oklahoma. The source of these copepods could not be determined; however, the species C. vernalis is known to be a common one in central Oklahoma (Kingsbury, 1968). A fifth culture was attempted at 5° C but was discontinued due to the low number of eggs produced and to the very slow rate of development of the immature stages. All animals used in experiments at 5°C were reared at 21°C and then transferred to the 5°C room at least 2 weeks before their use. The temperatures of 5, 14, and 21°C were maintained in constant temperature rooms, while the temperatures of 26 and 31°C were maintained in constant temperature chambers. Temperature was measured daily in one container at each temperature throughout the experiments and was never found to vary more than 1°C in any chamber or room. The culture medium consisted of pond water which had been filtered through a double layer of #12 bolting cloth (125 threads per inch). Each temperature room and chamber was set for alternating periods of 12-hours light and 12-hours dark. The cultures were fed the mixture of trout food and dried alfalfa whose preparation is described in Chapter 2. With this mixture a food source of constant quality could be provided at each temperature. The 1-liter cultures were fed 0.5 ml of food mixture twice each week. Observations of the copepods were made through a binocular microscope with magnifications of 7 to 30 times. Individual copepods were transferred using a micro-pipette.

Experimental Procedures

Late-stage immature female copepodites were selected from the stock cultures at 14°, 21°, 26°, and 31°C and placed singly in 4-dram shell vials, each of which contained 10 ml of filtered pond water. Two mature males were introduced and maintained in each vial from the time the female matured (adult molt) until her death. These males were always from the same stock culture as the female in whose vial they were placed. Each female was examined daily from the time she was mated until her death. The number of egg clutches produced by each female and the number of eggs in each clutch were recorded.

In order to reduce handling, egg counts were made with the egg sacs intact on the female. Counting of the eggs was facilitated by isolating the females in a small drop of water, thus restricting movement. No detrimental effect was noted for females handled by this method. Counting accuracy was checked occasionally by counting, in the usual way, the eggs on a gravid (egg-carrying) female taken from a stock culture; then removing the egg sacs and recounting after the egg sacs had been teased apart and the eggs were lying more or less in one plane. Some error was found in counts for the larger sacs of 30 or more eggs, but this error was small (not more than ± 3 eggs).

Nauplii were removed from a vial as soon as possible after a clutch of eggs hatched. Two drops of the food mixture were added to each vial twice per week, and the culture medium in each vial was replaced once per week.

To determine the development time for eggs at 14, 21, 26, and 31°C, each female in the egg production experiment, described above, was monitored at 8 hour intervals during a 4 week period. The development time for the eggs was estimated as the time from the first observation of the egg sac to the first time when the sac was no longer on the female. In any individual case this estimate may have been in error by as much as 8 hours, but on the average the differences should have largely balanced out and an unbiased mean value should have been obtained.

In addition to determining egg development at the above temperatures, egg development times were observed at 5°C. At this temperature several vials, each with mature female and 2 mature males taken originally from the 21°C cultures as previously mentioned, were examined once each day for several weeks, always at about the same time of day. The development times for egg sacs produced at this temperature were thus found within ± 1 day.

Data were also gathered on the influence of temperature on hatching success. Because of cannibalism (see Chapter 5), it was necessary, for this work, to remove the eggs from the female and to allow them to hatch while separated from her. Females carrying egg sacs were collected from the cultures at 14°, 21°, 26°, and 31°C and from the vials at 5°C. The egg sacs were removed from each female, the eggs were counted, and then the sacs were placed in a 5-cm (diameter) Petri dish containing 5 ml of filtered pond water. The eggs were checked at intervals of several hours, and the nauplii were counted as soon as possible after the eggs hatched.

DURATION OF THE FEMALE ADULT STAGE

The mean durations of the adult stage of Cyclops vernalis females were found to vary inversely with temperature (Table 53, Figure 17). The longest period that a female lived after its adult molt was 84 days and the shortest period was 20 days (Appendix G-1). The mean durations ranged from 26.2 days at 31°C to 74.2 days at 14°C. A significant difference ($P < 0.001$) was found among these means by analysis of variance. The homogeneity of the means was then tested by a posteriori comparisons (Student-Newman-Keuls test; Sokal and Rohlf, 1969). The results indicated that the duration of the adult stage was inversely related to temperature; the duration at each temperature being significantly longer ($P < 0.05$) than that observed at any of the higher temperatures and significantly shorter than that observed at any of the lower temperatures.

The mean time intervals between the adult molt and the production of the first egg sacs for females at the four temperatures were also compared (Table 53, Figure 17, Appendix G-1). When tested by analysis of variance, a significant difference ($P < 0.001$) was found among the mean intervals at the four temperatures. The time from the adult molt to the production of the first egg sacs was longest at 14°C, i.e. 27.3 days, and decreased with increasing temperatures; i.e. 23.9, 12.9,and 8.3 days at 21°, 26° and 31°C, respectively. From Figure 17 it can be seen that approximately one-third of the adult stage at each temperature was spent before egg production was initiated.

Table 53. MEAN DURATIONS OF THE ADULT STAGE, THE MEAN TIME INTERVALS FROM THE ADULT MOLT TO THE PRODUCTION OF THE FIRST EGG CLUTCH, THE MEAN TIMES FROM THE PRODUCTION OF THE LAST EGG CLUTCH TO DEATH, AND THE MEAN TIME INTERVALS BETWEEN SUCCESSIVE EGG CLUTCHES FOR CYCLOPS VERNALIS FEMALES AT FOUR DIFFERENT TEMPERATURES

Temperature (°C)	Times in days							
	Adult stage	$SE_{\bar{x}}$	Before 1st clutch	$SE_{\bar{x}}$	After last clutch	$SE_{\bar{x}}$	Between clutches	$SE_{\bar{x}}$
14	74.2	3.96	27.3	7.73	10.5	2.14	10.62	0.881
21	57.2	2.80	23.9	3.85	9.9	2.45	7.68	0.717
26	38.5	2.49	12.9	1.97	6.3	1.31	3.65	0.268
31	26.2	0.72	8.3	1.06	6.9	0.88	3.62	0.335

Coker (1934b) observed egg production beginning much earlier in the adult stage of *Cyclops vernalis* than reported in this paper. He found this period ranged from 7 days at 7 to 10°C to 3 days at 20 to 23°C. He also observed an increase in this interval at the highest temperature he studied, an increase to 7 days at 28 to 30°C. A similar result was not observed for the animals in the present study. Smyly (1970), in a study of the effect of diet on longevity of *Acanthocyclops viridis,* found that the interval from the adult molt to the initial egg clutch was 1 to 2 weeks for animals maintained on an animal diet and 4 weeks for animals fed algae only. The *C. vernalis* observed by Coker were fed protozoan infusions, whereas the animals observed for this paper were maintained on a diet which was composed mainly of plant material. The differences in diet composition may explain the discrepancy between the intervals preceding the production of the first egg clutch observed by Coker and those recorded in this paper.

The mean time between egg clutches decreased as temperature increased (Table 53, Figure 17, Appendix G-17). The mean intervals ranged from 3.62 days at 31°C to

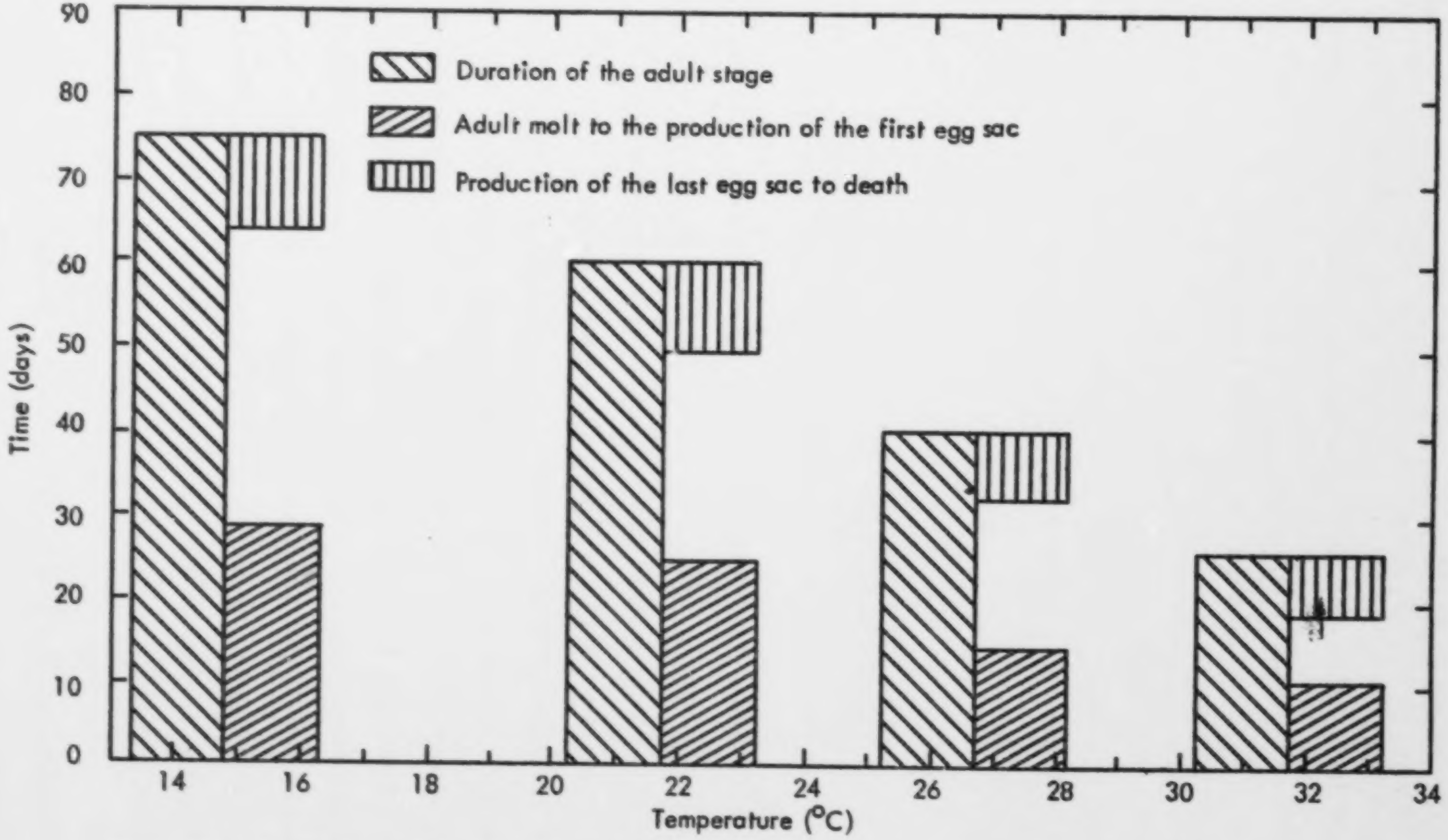

Figure 17. Comparison of the Mean Durations of the Adult Stage, the Mean Times From Maturation to the Production of the First Egg Clutch, and the Mean Times From the Production of the Last Egg Clutch to Death for C. vernalis Females at Different Temperatures.

10.62 days at 14°C. Clutches were produced every 3.65 and 7.68 days at 26° and 21°C, respectively. A significant difference among the means at the four temperatures was found at $P < 0.001$ using an anova test. Previous estimates of the time period between successive clutches are shorter than those reported here. Andrews (1953) found that C. vernalis, collected from Lake Erie and maintained in the laboratory at 20°C, produced new egg clutches every third day. Ewers (1936) observed that C. vernalis at room temperature produced eggs within 5 days following the adult molt and successive clutches could be produced at 1.5-day intervals. Again, it may be that a primarily vegetative diet slowed down the reproductive processes of the animals in the present study.

The data suggest that the period between the production of the last egg clutch and the death of the female is only slightly influenced by temperature (Table 53, Figure 17). Females died 1 to 37 days after the production of their last egg clutch (Appendix G-1). The longest mean interval (10.5 days) was observed at the lowest temperature of 14°C, and the shortest mean interval (6.3 days) was observed at 26°C. The interval at 21°C, i.e. 9.9 days, was only slightly longer than at 26°C. Analysis of variance showed no significant difference ($P > 0.05$) among these intervals at the four temperatures.

FECUNDITY

The reproductive histories of Cyclops vernalis females at 14°, 21°, 26°, and 31°C are shown in Appendices H-1 through H-4. These data are summarized in Table 54.

Clutch Size

Each C. vernalis female observed produced at least one egg sac. The clutches that were produced varied in size from 3 to 102 eggs. Both extremes were produced at 21°C. The variances for the mean clutch sizes at the four temperatures were significantly heterogeneous at $P < 0.05$ (Fmax test; Sokal and Rohlf, 1969), indicating that

Table 54. SUMMARY OF STATISTICAL DATA FOR CLUTCH SIZE, THE NUMBER OF CLUTCHES PRODUCED PER FEMALE, AND THE TOTAL EGG PRODUCTION PER FEMALE AT THE TEMPERATURES 14, 21, 26, AND 31°C.

Temperature (°C)	14	21	26	31
Clutch size (antilogs of log values)				
Mean	35.2	39.7	25.2	27.5
95% Confidence limits				
L_1	28.7	33.7	21.5	24.1
L_2	43.1	46.9	29.5	31.2
n	27	65	66	76
Min.	11	3	5	4
Max.	75	102	70	62
Number of clutches per female				
Mean	4.5	5.0	6.0	4.0
$SE_{\bar{x}}$	±0.81	±0.63	±0.77	±0.47
n	6	13	11	19
Min.	2	1	2	1
Max.	6	8	9	9
Total egg production per female				
Mean	177.8	233.9	180.9	123.9
$SE_{\bar{x}}$	±47.58	±30.44	±35.32	±19.94
n	6	13	11	19
Min.	36	3	25	4
Max.	310	388	430	356

the data did not meet the requirements for the anova test. In order to do such a test and ensuing a posteriori comparisons, the clutch size values were transformed to logarithms (base 10). All tests of significance were performed on the transformed data. The values for the mean clutch sizes discussed below are the antilogs of the means of the logarithmically transformed data. The greatest mean number of eggs per clutch was 39.7 eggs produced at 21°C, with the clutch size at 14°C only slightly less than this with 35.2 eggs. The least number of eggs per clutch was 25.2 eggs, produced at 26°C. The mean clutch size at 31°C was also quite low with only 27.5 eggs per clutch. Ewers (1936) and Armitage and Davis (1967) observed *C. vernalis* producing clutches of roughly the same size as those reported in this report (40 to 80 eggs per clutch and 16 to 23 eggs per sac, respectively). However, Andrews (1953) reported clutches ranging in size from 100 to 150 eggs.

Analysis of variance showed a significant difference ($P < 0.001$) among the means for clutch size at the four temperatures. *A posteriori* comparisons indicated that the mean size of egg clutches produced at 21°C was significantly larger ($P < 0.05$) than the means at either 26 or 31°C. Also, the mean size of the egg clutches produced at 14°C was significantly larger ($P < 0.05$) than that at 26°C. These results give some suggestion of an inverse relationship between clutch size and temperature, but the relation is not well defined by our data. Andrews (1953) reported a maximum rate of egg production by *C. vernalis* in the laboratory at the temperature of 21°C.

Several authors have suggested that the size of the egg clutches produced by cyclopoids is only indirectly related to temperature and that the factor directly determining clutch size is the female's body size. Margalef (1955) stated that the potential fecundity of cyclopoids is a function of body size and therefore of the temperature during development. Elbourn (1966) reported that the female's size and the temperature are equally important as factors determining the number of eggs produced per clutch by *C. strenuus strenuus*. The females' body sizes were not measured in the present experiments; however, Coker (1933) and Aycock (1942) published findings that show that the body size of C. vernalis is dependent on the temperature of development such that larger animals develop at lower temperatures. The fecundity data reported in this paper is for C. vernalis females which had developed at the temperature at which their egg production was observed. The variation of clutch size at the different temperatures may be the indirect result of the effect of temperature on body size. However, the data obtained are insufficient to verify this hypothesis.

Eggs produced by cyclopoid copepods are normally carried in paired egg sacs (Ewers, 1936; Wilson and Yeatman, 1959). However, during our experiments several females were observed carrying single egg sacs. One female (No. 11, Appendix H-3) was observed to carry only one egg sac in the majority of her clutches. If this female is excluded, most of the clutches consisting of only 1 egg sac were either the first, the last or the only egg clutch produced by that female. Although it may be that some of the single egg sac clutches were the result of one of the egg sacs being accidentally knocked off the female before the daily observations were made, most of the single egg sac clutches were probably due to a lower fecundity of the female at either the beginning or the end of her reproductive life.

To determine the effect that age has on fecundity at different temperatures, a series of comparisons were made using the Student's t-test. Tests were conducted to determine whether significant differences existed between the mean sizes of: (1) the first and last egg clutches, (2) the first clutches and all clutches and (3) the last clutches and all clutches. The mean size of the first egg clutches produced at a temperature was calculated by averaging the egg counts from the first and second clutches for all females at that temperature which produced at least four clutches during their lifetimes. The mean size for the last clutches was determined for each temperature by averaging the eggs counts from the last and next to last clutches for females which produced at least four egg clutches. The results (Table 55) show that clutch size was affected by the age of the female as well as by temperature. The mean size of the first

Table 55. CLUTCH SIZE IN RELATION TO AGE AND TO TEMPERATURE. THE VALUES FOR THE FIRST CLUTCHES ARE BASED ON THE AVERAGE OF THE FIRST AND SECOND CLUTCHES PRODUCED BY ALL FEMALES WHICH HAD FOUR OR MORE CLUTCHES. VALUES FOR THE LAST CLUTCHES ARE BASED ON THE AVERAGE OF THE NEXT TO LAST AND LAST CLUTCHES PRODUCED BY ALL FEMALES WHICH HAD FOUR OR MORE CLUTCHES. THE VALUES FOR ALL CLUTCHES ARE THE MEANS FOR ALL FEMALES WHICH HAD AT LEAST ONE EGG CLUTCH.

Temperature °C	Mean	$SE_{\bar{x}}$	n	Mean	$SE_{\bar{x}}$	n	t_s
	First Clutches			Last Clutches			
14	51.5	6.63	8	31.0	4.97	8	2.47*
21	55.8	4.61	18	32.5	3.74	18	3.95**
26	39.4	3.40	20	22.2	2.80	20	3.90***
31	39.3	2.34	22	22.5	1.91	22	5.55***
	First Clutches			All Clutches			
14	51.5	6.63	8	39.5	3.47	27	1.64ns
21	55.8	4.61	18	46.8	2.75	65	1.57ns
26	39.4	3.40	20	30.1	2.08	66	2.18*
31	39.3	2.34	22	31.0	1.52	76	2.68**
	Last Clutches			All Clutches			
14	31.0	4.97	8	39.5	3.46	27	1.23ns
21	32.5	3.74	18	46.8	2.75	65	2.55*
26	22.2	2.80	20	30.1	2.08	66	1.95ns
31	22.5	1.91	22	31.0	1.52	76	2.82**

ns Not significant at $P < 0.05$

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

*** Significant at $P < 0.001$.

clutches was found to be significantly different (14°C, $P < 0.05$; 21°C, $P < 0.01$; 26 and 31° C, $P < 0.001$) than the mean size of the last clutches at all temperatures, with the first clutches always being larger. At the higher temperatures, i.e. 26° and 31°C, the mean size of the first clutches was significantly different (26°C, $P < 0.05$; 31°C, $P < 0.01$) from the mean size for all clutches, again the first clutches were larger. At 21°C and 31°C the mean for the last clutches was different (21°C, $P < 0.05$; 31°C, $P < 0.01$) than the mean size for all clutches. Smyly (1970) observed that age affected clutch size in *Acanthocyclops viridis*, i.e. diminishing size with increasing age, and that diet affected the rate of this diminution. The experiments with *C. vernalis* also suggest that fecundity decreases as age increases.

Number of Clutches Per Female

Previous observations of *C. vernalis* indicated that females could produce a maximum of 12 egg clutches per lifetime (Ewers, 1936). Females in the experiments reported in this paper produced from a minimum of 1 to a maximum of 9 clutches per lifetime. The mean number of clutches produced per female varied little among the four temperatures studied (Table 54). The largest mean number of clutches (6.0) was produced by females at 26°C, and the smallest mean number of clutches (4.0) was produced by females at 31°C. No significant difference ($P > 0.05$) was found among the mean numbers of clutches produced at the four temperatures.

Total Egg Production

An anova showed the mean total number of eggs produced by a female during her lifetime varied among the four temperatures ($P < 0.05$). This would be expected since clutch size was found to vary with temperature, even though the number of clutches in a lifetime did not. Mean total egg production was highest at 21°C, i.e. 233.9 eggs, and lowest at 31°C, i.e. 123.9 eggs (Table 54). Means of egg production by females at 14° and 26°C were intermediate between the extremes and were very similar, 177.8 eggs at 14°C and 180.9 eggs at 26°C. Total egg production by females at 21°C was found by *a posteriori* comparison to be significantly greater than that by females at 31°C. All other paired comparisons of mean total egg production at the different temperatures proved to be not significant. The extremes for individual lifetime egg production ranged from 430 eggs produced by a female at 26°C to a low of 3 eggs contained in a single egg sac and produced by a female at 21°C.

DEVELOPMENT TIME OF EGGS

Cummins*et.* *al.*, (1969) considered the development period of copepod eggs as the period from the first appearance of eggs in the egg sacs to the time of hatching. In our experiments with *Cyclops vernalis,* the egg development rate was determined similarly.

The times for development of *C. vernalis* eggs in relation to temperature are shown in Table 56. For these data each clutch at 5 and 14°C was produced by a different female, whereas as many as 3 clutches may have been produced by a single female at the temperatures of 21°, 26°, and 31°C. The results indicate that the development times of eggs increase as temperature decreases. The mean development times, ranging from 28.4 hours at 31°C to 13.3 days at 5°C, were shown by analysis of variance to differ significantly ($P > 0.001$). *A posteriori* comparisons among the mean times at all the temperatures except 5°C indicated that the only two means not significantly different ($P \quad 0.05$) were those at 26 and 31°C. The development times at these temperatures were 31.3 hours and 28.4 hours, respectively. As the mean development time at 5°C was obviously much longer than the times at the higher temperatures, it was not included in the comparisons.

The rates of development of eggs, expressed as the reciprocal of mean development time, have been plotted against temperature (Figure 18). This plot shows a steady increase in the rate with temperature up to 26°C. From 26°C to 31°C the rate of increase of the development rate slows.

Table 56. DEVELOPMENT TIMES OF *CYCLOPS VERNALIS* EGG CLUTCHES AT DIFFERENT TEMPERATURES.

Clutch No.	Development Time (hours)				
	5° C	14° C	21° C	26° C	31° C
1	13 (days)	64	40	24	24
2	13 (days)	72	40	24	24
3	14 (days)	80	40	24	24
4		80	40	24	24
5		80	40	32	24
6			40	32	24
7			40	32	24
8			48	32	24
9			48	32	24
10			48	32	24
11			48	32	24
12				32	24
13				32	24
14				32	24
15				32	24
16				32	32
17				32	32
18				32	32
19				32	32
20				32	32
21				32	32
22				32	32
23				32	32
24				32	32
25				32	32
26				32	32
27				32	32
28				32	32
29				32	32
30				32	32
31				32	32
32				32	32
33				40	32
Mean	13.3	75.2	42.9	31.3	28.4
SE$_{\bar{x}}$		±3.20	±1.22	±0.54	±0.70
no.	3	5	11	33	33

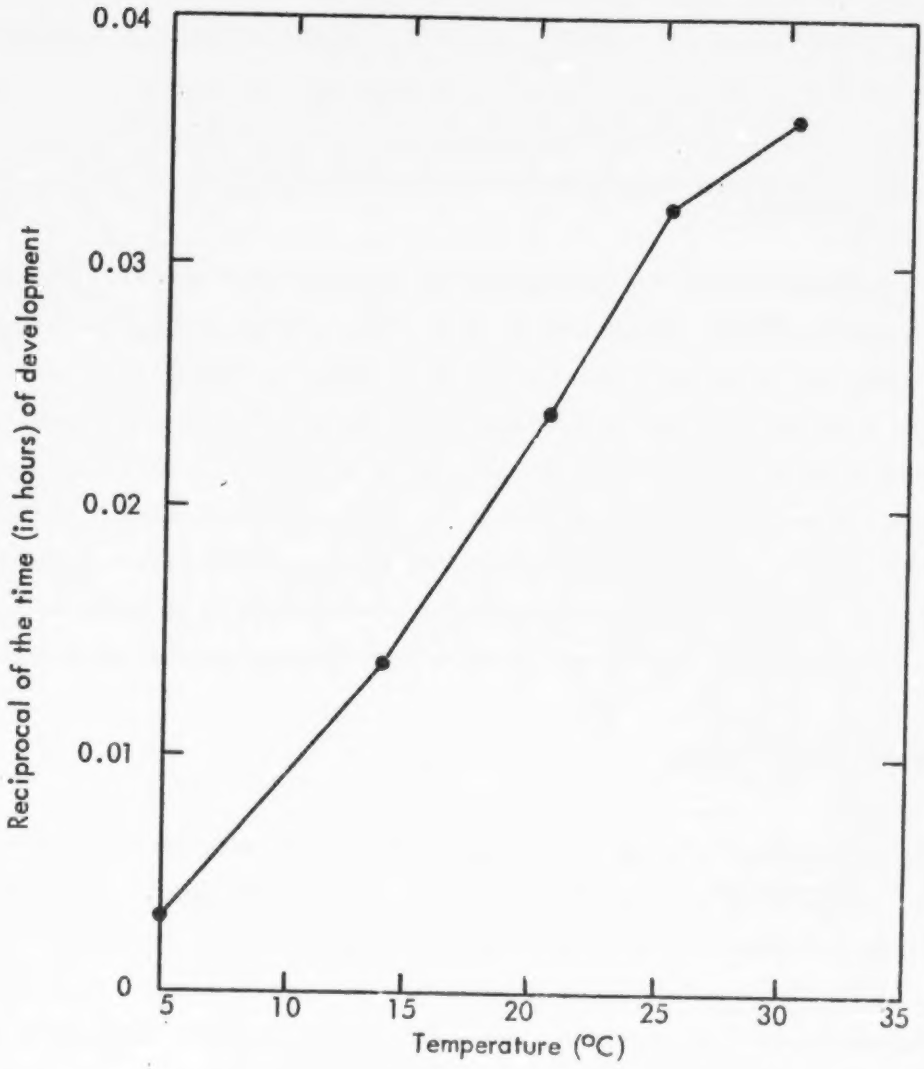

Figure 18. The Rate of Development (Expressed as the Reciprocal of Development Time in Hours) of Eggs of *Cyclops vernalis* in Relation to Temperature.

Several authors, Elbourn (1966), Khan (1965), and Cummins *et. al.*, (1969), have observed an increase in development rate of cyclopoid eggs with increasing temperature. On the other hand, Burgis (1970) reported that the development rate of the eggs of the tropical cyclopoid *Thermocyclops neglectus* was retarded at temperatures above 32.5°C. That the development rate of *Cyclops vernalis* eggs also would probably be retarded at higher temperatures is suggested by the decrease in the rate of increase of the development rate that was observed between 26 and 31°C.

A correlation between egg development rate and the interval between successive clutches at different temperatures was noted for *C. strenuus strenuus* by Elgmork (1959). Similar results have been found for *C. vernalis.* Both the development rate of the eggs and the period between successive egg clutches are inversely related to temperature. The duration that egg sacs are carried by female *C. vernalis* is dependent on the development rate of the eggs (the sacs are normally carried until the eggs hatch). Possibly the carrying of the egg sacs inhibits further egg production. If this is true, the development rate of eggs, as determined by temperature, would to some extent determine the rate at which successive egg clutches are produced.

HATCHING SUCCESS

The percentage of the eggs that hatched in the clutches examined ranged from 75.4% to 100% (Table 57). Over 90% hatching success was quite common. The mean percentages of hatching at the five temperatures ranged from 88.1% at 5°C to 96.0% at 26°C. An analysis of variance showed no significant differences ($P > 0.05$) among the mean percentages of hatching at the different temperatures. The method used in removing the eggs from the females and in counting the eggs may have resulted in injury to a few eggs. If this is true, the percentage of hatching under natural conditions may be even closer to 100% than is indicated in this study.

Table 57. SUCCESS OF HATCHING FOR EGG CLUTCHES PRODUCED BY CYCLOPS VERNALIS IN RELATION TO TEMPERATURE.

Clutch No.	5°C			14°C			21°C			26°C			31°C		
	Eggs	Nauplii	% hatch	Eggs	Nauplii	% hatch	Eggs	Nauplii	% hatch	Eggs	Nauplii	% hatch	Eggs	Nauplii	% hatch
1	59	52	88.1	45	43	95.5	44	40	90.0	59	58	98.3	78	72	92.3
2	47	44	93.6	60	56	93.3	60	58	96.6	85	84	98.8	71	70	98.6
3	80	66	82.5	63	55	87.3	70	62	88.6	66	63	95.5	67	65	97.0
4				59	51	86.4	89	75	84.3	68	65	95.6	56	54	96.4
5				72	69	95.8	60	50	83.3	48	46	95.8	67	62	92.5
6				77	72	93.5	82	71	86.6	73	69	94.5	77	60	77.9
7				42	40	95.2	66	66	100.0	82	76	92.7	70	62	88.6
8				41	38	92.7	63	60	95.2	32	31	96.9	51	50	98.0
9				44	35	79.5	102	97	95.1				42	40	95.2
10				34	34	100.0	60	60	100.0				82	78	95.1
11				67	62	92.5	124	117	94.3				65	60	92.3
12				55	50	90.9	110	105	95.5				75	65	86.7
13				43	38	88.4	36	32	88.8				78	74	94.9
14				33	30	90.9	140	124	88.6						
15				72	68	94.4	80	78	97.5						
16				114	86	75.4	43	43	100.0						
17				90	89	98.8	107	99	92.5						
18				87	87	100.0	63	59	93.7						
19				70	65	92.9	74	66	89.2						
20				34	30	88.2	54	50	92.6						
21							97	88	90.7						
22							68	67	98.5						
Mean			88.1			91.6			92.8			96.0			92.7
$SE_{\bar{x}}$			±3.20			±1.39			±1.06			±0.70			±1.57
No.			3			20			22			8			13

The results in the present study agree generally with the findings of Walter (1922) in work with *Cyclops viridis* and Elbourn (1966) in work with *C. strenuus strenuus*, both of whom also found that the hatching success of cyclopoid eggs was not affected by temperature. However, Elbourn reported hatching success of only 60 to 80% at all temperatures, whereas Walter reported approximately 80 to 90% success. Khan (1965) reported decreasing hatching success of eggs of *Acanthocyclops viridis* at temperatures above 26°C but did not elaborate on the extent of the egg mortality. Burgis (1970) observed that all eggs of the tropical cyclopoid *Thermocyclops neglectus* failed to hatch at temperatures above 35°C.

SUMMARY

The effects of temperature on certain of the reproductive attributes of the cyclopoid copepod *Cyclops vernalis* have been studied. Four temperatures, 14°, 21°, 26°, and 31°C, were used in all experiments and a fifth, 5°C, in some. The following conclusions were reached:

1. The mean duration of the adult stage of the females is inversely related to temperature.

2. The mean time interval from the adult molt to the start of egg laying and the time between successive clutches are both also inversely related to temperature. The time from the last clutch to death did not seem to be significantly affected by temperature, however.

3. Clutch size was found to be significantly different among temperatures, with a tendency for the animals at 21 and 14°C to have larger clutch sizes than the animals at 26 and 31°C.

4. Temperature was not, however, related to the number of clutches produced by a female during her lifetime.

5. As expected from the combined effects of temperature on clutch size and number, a significant inverse relation was found between total lifetime production of eggs and temperature.

6. A female's clutch size is related to her age. The earlier clutches laid by a female tended to be larger than the later ones.

7. Egg development rate is inversely related to temperature.

8. No significant differences could be detected in the percent of the eggs that hatched at the various temperatures.

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APPENDIX A-1

14°C INCUBATION: NUMBER OF EGGS PER CLUTCH IN THE SEQUENCE PRODUCED

Female's hatching temperature	14°	21°	31°			Total	Mean	±Standard error
Individual	A7	A6	A8	A9	A10			
Clutch No.								
1	30	24	20	16	23	113	22.6	2.32
2	26	18	23	18	30	115	23.0	2.32
3	23	19	19	14	22	97	19.4	1.57
4	22	15	22	16	21	96	19.2	1.53
5	26	18	13	24	24	105	21.0	2.41
6	31	19	24	18	24	116	23.2	2.31
7	21	15		10	22			
8	30	15						
9	24	15						
10	34							
11	28							
12	27							
Total	322	158	121	116	166			
Mean	26.8	17.6	20.2	16.6	23.7			
Standard error	1.14	1.00	1.62	1.62	1.13			

Anova table: mean clutch size

Source of variation	df	MS	F	
Among Individuals	4	174.0824	12.8986	P< 0.001
A7 vs Others	1	475.9767	35.2675	P< 0.001
A6 vs 31° hatch	1	41.7795	3.0956	ns
A8 vs A9 vs A10	2	89.2869	6.6157	P< 0.050
Within individuals	36	13.4962		
Total	40			

Anova table: comparison of sequential clutches

Source of variation	df	MS	F	
Among sequential clutches	5	16.2400	0.7298	ns
Within sequential clutches	24	22.2500		
Total	29			

APPENDIX A-2

21° INCUBATION: NUMBER OF EGGS PER CLUTCH IN THE SEQUENCE PRODUCED

Female's hatching temperature	21°							27°	31°		Total	Mean	±Standard error
Individual	B11	B12	B14	B17	B18	B19	B20	B16	B13	B15			
Clutch No.													
1	25	27	22	24	28	22	27	18	14	18	225	22.5	1.46
2	29	28	16	30	22	38	(28)	20	24	12	247	24.7	2.39
3	42	19	18	19	30	26	35	23	01	11	224	22.4	3.72
4	45	32		25	25	35	23	18	15	12	230	25.6	3.47
5	21	31		42	40	25	26	32	19	27	263	29.2	2.62
6	22	27		17	43	33	43	14	21	24	244	27.1	3.51
7	28	26			19	25	35	17	27	18	195	24.4	2.15
8	28	33			22		19	13	29	11	155	22.1	3.15
9	25	30					28	24	19	17	143	23.8	2.06
10	21	35					27	25	14	13	135	22.5	3.40
11	21	42					32	15	14	09	133	22.2	5.11
12	27	32					27	17	16	08	127	21.2	3.66
13	16	27					08	28		05	84	16.8	4.73
14	22	28						34		12			
15	30	26								14			
16	29	34								24			
17	16	44								17			
18	20	34								13			
19	20	36											
20	26	35											
21	28	35											
22	40	34											
23	32	30											
24	16	24											
25		25											
Total	629	774	56	157	229	204	358	298	213	265			
Mean	26.2	31.0	18.7	26.2	28.6	29.1	27.5	21.3	17.8	14.7			
Standard error	1.58	1.11	1.76	3.68	3.08	2.30	2.33	1.76	2.13	1.38			

Parentheses indicate a clutch which was not counted. Value is an average for that female.

APPENDIX A-2 (Cont.)

21° INCUBATION: ANALYSES OF VARIANCE

Anova table: mean clutch sizes

Source of variation	df	MS	F	
Among individuals	9	450.1521	9.1601	P< 0.001
21° hatch vs other	1	3119.2990	63.4747	P< 0.001
27° hatch vs 31° hatch	1	273.4580	5.5646	P< 0.050
B13 vs B15	1	66.0056	1.3431	ns
Among 21° hatch	6	98.7679	2.0098	ns
Within individuals	120	49.1424		
Total	129			

Anova table: comparison of sequential clutches

Source of variation	df	MS	F	
Among sequential clutches	12	64.1855	0.8088	ns
Within sequential clutches	88	79.3545		
Total	100			

APPENDIX A-3

27° INCUBATION: NUMBER OF EGGS PER CLUTCH IN THE SEQUENCE PRODUCED

Female's hatching temperature	21°	27°						31°	Total	Mean	±Standard error
Individual	C9	C7	C8	C13	C14	C15	C16	C11			
Clutch No.											
1	19	35	29	32	27	20	04	15	181	22.6	3.60
2	18	36	45	35	22	29	07	20	212	26.5	4.28
3	18	40	45	42	27	27	25	30	254	31.8	3.36
4	23	26	36	33	21	29	13	36	217	27.1	2.84
5	20	29	47	38	22	26	09	32	223	27.9	4.10
6	20	39	41	34	24	32		35	225	32.1	2.89
7	25	34	45	33	25	32		13	207	29.6	3.75
8		43	50	27	35	30			185	37.0	4.23
9		19	33	23		29					
10		22	34	22		25					
11		32	36								
12		42	46								
13		17	21								
14		25	34								
15		26	32								
16		20	36								
17		20	45								
18			21								
Total	143	505	676	319	203	279	58	181			
Mean	20.4	29.7	37.6	31.9	25.4	27.9	11.6	25.9			
Standard error	1.00	2.09	2.02	1.99	1.59	1.14	3.66	3.65			

APPENDIX A-3 (Cont.)

27° INCUBATION: ANALYSES OF VARIANCE

Anova table: mean clutch sizes

Source of variation	df	MS	F	
Among individuals	7	517.4556	9.7579	$P < 0.001$
27° hatch vs other	1	545.8955	10.2962	$P < 0.005$
21° hatch vs 31° hatch	1	103.1429	1.9454	ns
Among 27° hatch	5	594.6302	11.2154	$P < 0.005$
Within individuals	74	53.0191		
Total	81			

Anova table: comparison of sequential clutches

Source of variation	df	MS	F	
Among sequential clutches	7	123.4961	1.2403	ns
Within sequential clutches	51	99.5626		
Total	58			

APPENDIX A–4

31° INCUBATION: NUMBER OF EGGS PER CLUTCH IN THE SEQUENCE PRODUCED

Female's hatching temperature	21°	27°	31°								
Individual	D8	D5	D3	D11	D16	D17	D19	D20	Total	Mean	±Standard error
Clutch No.											
1	11	30	20	29	27	24	25	17	183	22.9	2.29
2	12	34	(17)	20	33		27	21	164	23.4	3.11
3	13	26	21	29	26		22	13	150	21.4	2.40
4	18	26	11	41	26		20	12	154	22.0	3.89
5	14			28	(25)		14	12	93	18.6	3.28
6					25		23	13			
7					13			10			
8					31			10			
9					19						
Total	68	116	69	147	225	24	131	108			
Mean	13.6	29.0	17.2	29.4	25.0	24.0	21.8	13.5			
Standard error	1.21	1.91	2.25	3.36	1.99		1.85	1.32			

Parentheses indicate a clutch which was not counted. Number in parentheses is an average value for that female.

Anova table: mean clutch size

Source of variation	df	MS	F	
Among individuals	7	220.7370	8.8714	$P < 0.001$
31° hatch vs other	1	5.5873	0.2246	ns
D8 vs D5	1	527.0222	21.1810	$P < 0.001$
D3 vs D11 vs ... D20	5	202.5100	8.1389	$P < 0.001$
Within individuals	34	24.8818		
Total	41			

Anova table: comparison of sequential clutches

Source of variation	df	MS	F	
Among sequential clutches	4	20.0064	0.3238	ns
Within sequential clutches	29	61.7759		
Total	33			

APPENDIX B-1

14° INCUBATION: TIME (HOURS) CLUTCHES WERE CARRIED

Female's hatching temperature	14°	21°	31°		
Individual	A7	A6	A8	A9	A10
	107.5	120.0	119.5	115.5	115.5
	114.0	116.5	104.5	121.0	96.5
	127.0	114.5	97.5	114.5	107.5
	132.5	123.0	152.0	111.0	124.5
	119.5	106.5	117.0		113.5
	119.5	109.5	97.5		120.5
	120.0	114.0			
	107.0	118.5			
	112.0	65.0			
	113.0				

Clutch carrying times are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some carrying times having been omitted for various reasons as discussed in the text.

Variances of the data for the three hatching temperatures are marginally homogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: comparison among individuals of carrying times.

Source of Variation	df	MS	F	
Among individuals	4	0.0014	0.4242	ns
Within individuals	30	0.0033		
Total	34			

APPENDIX B-2

21° INCUBATION: TIME (HOURS) CLUTCHES WERE CARRIED

Female's hatching temperature	21°							27°	31°	
Individual	B11	B12	B14	B17	B18	B19	B20	B16	B13	B15
	53.5	46.5	49.0	49.0	52.5	55.5	47.0	53.0	48.0	24.0
	47.0	47.0	49.5	49.0	49.5	45.0	49.5	49.0	44.0	44.5
	56.5	38.0*	60.0	54.0	54.0	46.5	52.5	57.5	10.0	47.0
	55.5	36.0*		53.5	50.5	52.5	50.5	46.0	58.0	56.0
	42.5	43.0*		46.0	41.0	54.0	53.5	51.0	43.5*	44.5
	54.5	45.5*		54.5	31.0*	50.0	48.0	51.0	47.0*	57.5
	60.0	31.5*			31.5*		55.5	48.0	49.0*	46.5
	39.0	43.0*			52.0		53.5	41.5	40.0*	45.0
	43.5	46.5*					54.5	54.5	44.0*	39.0
	44.5	45.0*					48.5	47.0	63.5*	72.0
	45.5	36.5*					52.5	56.0	44.5*	52.5
	68.5	40.5*					56.5	51.0	36.5*	71.5
	23.5	24.0*					50.5	48.0		45.0
	52.0	43.0								52.0
	43.0	45.0								65.0
	64.5	24.0*								
	36.5	47.5*								
	22.5	39.0*								

*Indicates carrying time of a clutch of resting eggs.

APPENDIX B-2 (Cont.)

21° INCUBATION: TIME (HOURS) CLUTCHES WERE CARRIED

Clutch carrying times are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some carrying times having been omitted for various reasons as discussed in the text.

Variances of the data for the three hatching temperatures are heterogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: carrying times of resting eggs (mean = 39.0) vs carrying times of non-resting eggs (mean = 48.2).

Source of Variation	df	MS	F	
Among egg types	1	0.1755	15.2608	P<0.001
Within egg types	113	0.0115		
Total	114			

Anova table: comparison among individuals of carrying times, excluding those times marked with an asterisk above which are the carrying times of clutches of resting eggs.

Source of Variation	df	MS	F	
Among individuals	9	0.0160	1.3793	ns
Within individuals	78	0.0116		
Total	87			

APPENDIX B-3

27° INCUBATION: TIME (HOURS) CLUTCHES WERE CARRIED

Female's hatching temperature	21°	27°						31°
Individual	C9	C7	C8	C13	C14	C15	C16	C11
	45.5	41.5	35.5	40.0	40.5	44.5	26.0	25.0
	25.5	43.0	19.5	35.5	37.0	38.0	28.0	38.0
	45.0	40.0	33.5	34.5	34.5	30.5	17.5	36.0
	30.5	37.0	33.5	27.5	27.5	34.5	10.0	34.5
	33.0	37.0	42.5	35.5	35.0	32.0		
	35.0	33.0	35.5	42.0	32.0	35.0		
		32.5	36.5	38.5	35.0	39.0		
		35.0	25.0	39.5	44.0			
		35.5	23.5	43.5				
		24.0	34.0	44.0				
		24.0	30.5					
		18.5	23.5					
		24.0	20.5					
			41.0					
			52.5					

Clutch carrying times are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some carrying times having been omitted for various reasons as discussed in the text.

Variances of the data for the three hatching temperatures are marginally homogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: comparison among individuals of carrying times.

Source of Variation	df	MS	F	
Among individuals	7	0.0427	3.9537	$P<0.010$
27° hatch vs other	1	0.0045	0.4170	ns
21° hatch vs 31° hatch	1	0.0016	0.1519	ns
C7 vs C8 vs ... vs C16	5	0.0587	5.4380	$P<0.001$
Within individuals	59	0.0108		
Total	66			

APPENDIX B-4

31° INCUBATION: TIME (HOURS) CLUTCHES WERE CARRIED

Female's hatching temperature	21°	27°	31°					
Individual	D8	D5	D3	D11	D16	D17	D19	D20
	25.0	22.5	24.5	37.5	30.5	23.5	29.5	30.0
	35.0	24.0		37.0	28.5		37.0	22.5
	34.0	24.0			34.5		35.5	38.5
	36.0	35.0			23.5		31.0	28.0
	35.5				28.0		25.5	32.5
					23.0		29.0	32.0
					29.5			35.0
								30.0

Clutch carrying times are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some carrying times having been omitted for various reasons as discussed in the text.

Variances of the data for the three hatching temperatures are marginally homogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: comparison among individuals of carrying times.

Source of Variation	df	MS	F	
Among individuals	7	0.0097	2.2045	ns
Within individuals	26	0.0044		
Total	33			

APPENDIX B-5

14° INCUBATION: INTERVAL (HOURS) BETWEEN CLUTCHES

Female's hatching temperature	14°	21°	31°		
Individual	A7	A6	A8	A9	A10
	13.0	60.5	48.0	24.0	25.0
	5.5	8.0	25.0	47.5	8.0
	24.0	20.5	24.5	8.5	93.0
	151.0	60.0	19.5	185.5	
	49.5	59.0			
	14.0	170.0			
	39.5	9.0			
	352.0	99.5			
		96.0			

Intervals between successive clutches are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some intervals having been omitted for various reasons as discussed in the text.

Variances of the data for the three hatching temperatures are marginally homogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: comparison among individuals of intervals between clutches.

Source of Variation	df	MS	F	
Among individuals	4	0.0404	0.1596	ns
Within individuals	23	0.2530		
Total	27			

APPENDIX B-6

21° INCUBATION: INTERVAL (HOURS) BETWEEN CLUTCHES

Female's hatching temperature	21°							27°	31°	
Individual	B11	B12	B14	B17	B18	B19	B20	B16	B13	B15
	20.0	15.0	10.0	23.0	8.5	7.5	9.5	7.5	5.5	36.5
	5.0	3.5	23.5	23.5	14.0	12.0	5.0	11.5	122.0	15.0
	3.5	11.0*		82.5	57.5	3.0	11.0	3.5	38.0	4.0
	5.0	3.5*		3.0	5.5	58.0	7.0	10.5	3.0	5.5
	4.5	3.0*		4.0	11.0	3.5	3.0	8.0	3.5*	12.0
	2.5	3.0*			47.0*	21.5	8.0	24.5	3.0*	5.0
	11.5	4.5*			60.5*		97.5	9.5	8.0*	18.5
	8.5	5.0*					5.0	6.0	7.5*	8.0
	23.0	18.5*					109.0	25.0	52.0*	8.0
	8.0	8.0*					8.0	8.0	7.5*	44.5
	5.5	8.0*					51.5	6.0	24.0*	16.0
	8.0	8.0*					29.5	7.5		7.5
	16.0	24.5*								70.5
	24.0	23.5								4.0
	7.5	8.0								3.5
	8.0	16.0*								
	4.0	8.0*								
	12.5	7.5*								
		7.5*								
		8.5*								
		25.0*								
		4.5								

*Indicates an interval following a clutch of resting eggs.

APPENDIX B-6 (Cont.)

21° INCUBATION: INTERVAL (HOURS) BETWEEN CLUTCHES

Intervals between successive clutches are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some intervals having been omitted for various reasons as discussed in the text.

Variances of the data for the three hatching temperatures are heterogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: intervals following clutches of resting eggs (mean = 9.7) vs intervals following clutches of non-resting eggs (mean = 10.6).

Source of Variation	df	MS	F	
Among interval types	1	0.0284	0.1762	ns
Within interval types	107	0.1611		
Total	108			

Anova table: comparison among individuals of intervals between clutches, excluding those intervals marked with an asterisk above which followed a clutch of resting eggs.

Source of Variation	df	MS	F	
Among individuals	9	0.0969	0.5601	ns
Within individuals	74	0.1730		
Total	83			

APPENDIX B-7

27° INCUBATION: INTERVAL (HOURS) BETWEEN CLUTCHES

Female's hatching temperature	21°	27°						31°
individual	C9	C7	C8	C13	C14	C15	C16	C11
	11.5	8.0	12.5	11.0	9.0	4.0	24.0	10.0
	4.5	8.5	25.0	4.0	3.5	18.0	11.0	3.5
	4.5	8.0	17.0	22.5	22.5	18.5	4.5	22.5
	12.0	11.0	4.5	8.5	9.0	9.5	168.0	12.5
	4.5	12.5	4.0	3.5	3.5	19.5		
		12.0	12.5	4.5	4.0	10.0		
		5.5	12.5	10.0	8.0			
		25.5	12.0	7.0				
		12.5	24.0	5.5				
		37.5	24.0					
		97.0	39.0					
		172.0	12.5					
			24.0					
			7.5					
			24.5					

Intervals between successive clutches are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some intervals having been omitted for various reasons as discussed in the text.

Variances of the data for the three harching temperatures are heterogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: comparison among individuals of intervals between clutches.

Source of Variation	df	MS	F	
Among individuals	7	0.2682	2.1370	ns
Within individuals	54	0.1255		
Total	61			

APPENDIX B-8

31° INCUBATION: INTERVAL (HOURS) BETWEEN CLUTCHES

Female's hatching temperature	21°	27°	31°					
Individual	D8	D5	D3	D11	D16	D17	D19	D20
	22.0 13.5 13.0 61.0	24.0 24.5 24.0	191.5 12.0	10.5 9.5	17.5 19.0 81.5 24.0 21.5 96.0	-----	11.0 14.0 33.0 24.0 4.5	49.5 18.0 9.0 22.0 38.0 31.0 22.5

Intervals between successive clutches are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some having been omitted for various reasons as discussed in the text.

Variances of the data for the three hatching temperatures are heterogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: comparison among individuals of intervals between clutches.

Source of Variation	df	MS	F	
Among individuals	6	0.1420	1.3172	ns
Within individuals	22	0.1078		
Total	28			

APPENDIX B-9

14° INCUBATION
EGG PRODUCTION CYCLE

Time (hours) from oviposition to succeeding oviposition

Female's hatching temperature	14°	21°	31°		
Individual	A7	A6	A8	A9	A10
	168.0	133.0	167.5	139.5	139.0
	122.0	122.0	129.5	166.5	227.5
	147.5	138.5	122.0	168.5	132.0
	192.5	272.0	136.5	123.0	132.5
	279.5	156.0		296.5	206.5
	129.0	123.5			
	206.5	153.5			
	208.0	470.5			

Lengths of cycles are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some cycles having been omitted for various reasons as discussed in the text.

Variances of the data for the three hatching temperatures are marginally homogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: comparison among individuals of complete egg production cycle.

Source of Variation	df	MS	F	
Among individuals	4	0.0087	0.3954	ns
Within individuals	25	0.0220		
Total	29			

APPENDIX B-10

21° INCUBATION
EGG PRODUCTION CYCLE
Time (hours) from oviposition to succeeding oviposition

Female's hatching temperature	21°							27°	31°	
Individual	B11	B12	B14	B17	B18	B19	B20	B16	B13	B15
	73.5	61.5	59.0	72.0	61.0	63.0	56.5	59.0	53.5	60.5
	50.5	50.5	73.0	72.5	63.5	57.0	54.5	60.5	166.0	59.5
	47.0	55.5		136.5	111.5	49.5	63.5	60.5	48.0	61.0
	52.0	49.0*		56.5	56.0	110.5	57.0	61.0	61.0	61.5
	60.0	39.5*		50.5	52.0	57.5	56.5	56.5	47.0*	56.5
	60.0	46.0*			78.0*	71.5	56.0	59.0	50.0*	62.5
	47.0	48.5*			92.0*		153.0	75.5	57.0*	65.0
	57.0	36.0*					58.5	57.5	47.5*	53.0
	71.5	48.0*					163.5	47.5	96.0*	47.0
	47.5	65.0*					56.5	79.5	71.0*	116.5
	66.5	53.0*					104.0	55.0	68.5*	71.0
	52.5	46.5*					86.0	62.0		68.5
	51.0	48.5*						58.5		79.0
	40.0	48.5*								115.5
	76.5	66.5								56.0
	39.5	53.0								68.5
	76.0	40.0*								
	50.5	55.5*								
	72.5	46.5*								
	40.5	50.0*								
		46.5*								
		50.0*								

*Indicates a cycle which includes a clutch of resting eggs.

APPENDIX B-10 (Cont.)

21° INCUBATION
EGG PRODUCTION CYCLE
Time (hours) from oviposition to succeeding oviposition

Lengths of cycles are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some cycles having been omitted for various reasons as discussed in the text.

Variances of the data for the three hatching temperatures are heterogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: cycles including clutches of resting eggs (mean = 53.2) vs cycles including clutches of non-resting eggs (mean = 63.7).

Source of Variation	df	MS	F	
Among cycle types	1	0.1248	8.5479	P<0.005
Within cycle types	112	0.0146		
Total	113			

Anova table: comparison among individuals of complete egg production cycles excluding those cycles marked with an asterisk above which included a clutch of resting eggs.

Source of Variation	df	MS	F	
Among individuals	9	0.0196	1.2980	ns
Within individuals	78	0.0151		
Total	87			

APPENDIX B-11

27° INCUBATION
EGG PRODUCTION CYCLE

Time (hours) from oviposition to succeeding oviposition

Female's hatching temperature	21°	27°						31°
Individual	C9	C7	C8	C13	C14	C15	C16	C11
	57.0	50.0	48.0	51.0	49.5	48.5	50.0	35.0
	39.5	45.5	36.5	39.0	40.5	39.5	39.0	41.5
	30.0	51.0	38.0	57.0	57.0	56.0	22.0	58.5
	49.5	48.0	37.5	36.0	36.5	49.0	178.0	48.0
	42.5	44.0	55.0	39.0	38.5	36.0		47.0
	37.5	51.0	48.0	46.5	36.0	44.0		48.5
		25.0	48.5	48.5	43.0	55.0		
		48.0	49.0	46.5		51.5		
		49.5	47.5	49.0		45.5		
		45.0	73.0					
		38.0	43.0					
		60.5	47.5					
		48.0	28.0					
		61.5	65.5					
		121.0						
		190.5						

Lengths of cycles are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some cycles having been omitted for various reasons as discussed in the text.

Variances of the data for the three hatching temperatures are heterogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: comparison among individuals of complete egg production cycles.

Source of Variation	df	MS	F	
Among individuals	7	0.0141	0.6588	ns
Within individuals	63	0.0214		
Total	70			

APPENDIX B-12

31° INCUBATION
EGG PRODUCTION CYCLE
Time (hours) from oviposition to succeeding oviposition

Female's hatching temperature	21°	27°	31°					
Individual	D8	D5	D3	D11	D16	D17	D19	D20
	47.0 48.5 47.0 97.0	46.5 48.5 48.0	36.5	48.0 24.5 46.5 96.0	48.0 97.5 47.5 116.0 76.0 47.5 49.5 119.0	-----	40.5 51.0 68.5 55.0 30.0	79.5 40.5 47.5 50.0 70.5 63.5 57.5

Lengths of cycles are listed from top to bottom in the order of occurrence but are not necessarily consecutive, some cycles having been omitted for various reasons as discussed in the text.

Variances of the data for the three hatching temperatures are heterogeneous. A logarithmic transformation was made and Anovas carried out with the transformed data.

Anova table: comparison among individuals of complete egg production cycle.

Source of Variation	df	MS	F	
Among individuals	6	0.0276	1.1219	ns
Within individuals	25	0.0246		
Total	31			

APPENDIX C-1

14° INCUBATION: PERCENT HATCH

Female's hatching temperature	14°	21°	31°		
Individual	A7	A6	A8	A9	A10
	100.0%	45.8%	15.0%	87.5%	100.0%
	100.0	61.1	4.4	100.0	100.0
	82.6	78.9	0.0	64.3	95.5
	45.5	73.3	22.7	56.2	81.0
	100.0	94.4	46.2	50.0	95.8
	74.2	57.9	0.0	27.8	70.8
	85.7	33.3		0.0	81.8
	93.3	66.7			
	83.3	86.7			
	61.8				
	85.7				
	7.4				
Mean %	82.3%	68.0%	9.2%	55.7%	92.9%

An arcsine transformation of the data above was made, the means above were computed, and an Anova carried out:

Source of Variation	df	MS	F	
Among individuals	4	3146.0922	8.2802	P<0.001
A7 vs Others	1	1797.1592	47.3521	P<0.001
A6 vs A8, 9 & 10	1	325.3057	0.8562	ns
A8 vs A9 vs A10	2	5230.9521	13.7674	P<0.001
Within individuals	36	379.9531		
Total	40			

APPENDIX C-2

21° INCUBATION: PERCENT HATCH

Female's hatching temperature	21°							27°	31°	
Individual	B11	B12	B14	B17	B18	B19	B20	B16	B13	B15
	24.0%	74.1%	81.8%	91.7%	92.2%	86.4%	96.3%	100.0%	14.3%	100.0%
	100.0	100.0	37.5	83.3	59.1	86.8	88.6	85.0	70.8	25.0
	95.2	100.0	0.0	100.0	73.3	88.5	95.7	91.3	0.0	54.5
	46.7	65.4		95.2	96.0	71.4	100.0	77.8	60.0	75.0
	23.8	20.6		88.2	22.5	96.0	79.1	100.0		85.2
	63.6	60.0			72.7	66.7	94.3	92.9		100.0
	100.0					100.0	68.4	88.2		100.0
	89.3						89.3	92.3		81.8
	100.0						100.0	87.5		94.1
	42.9						93.8	92.0		53.8
	100.0						96.3	100.0		100.0
	37.0						100.0	100.0		3.8
	100.0							100.0		100.0
	95.5							100.0		75.0
	70.0									92.9
	100.0									95.8
	87.5									76.5
	100.0									100.0
	90.0									
	100.0									
	100.0									
Mean %	88.1%	77.7%	31.6%	93.5%	72.2%	88.0%	94.5%	96.2%	29.0%	85.5%

APPENDIX C-2 (Cont.)

21° INCUBATION: PERCENT HATCH

An arcsine transformation of the data on the previous page was made, the means on the previous page were computed, and an Anova carried out:

Source of Variation	df	MS	F	
Among individuals	9	1345.5168	3.6499	$P<0.001$
B11, B12, ..., B20 vs Others	1	1.2514	0.0034	ns
B16 vs B13 & B15	1	2627.9419	7.1287	$P<0.010$
B13 vs B15	1	4027.4391	10.9251	$P<0.010$
B11 vs B12 vs .... vs B20	6	908.8366	2.4654	$P<0.050$
Within individuals	86	368.6425		
Total	95			

APPENDIX C-3

27° INCUBATION: PERCENT HATCH

Female's hatching temperature	21°	27°						31°
Individual	C9	C7	C8	C13	C14	C15	C16	C11
	0.0%	54.3%	44.8%	87.5%	100.0%	65.0%	0.0%	13.3%
	86.7	100.0	20.0	48.6	100.0	82.8	42.9	45.0
	27.8	12.5	62.2	76.2	85.2	96.3	76.0	6.7
	43.5	0.0	44.4	81.8	100.0	79.3	100.0	61.1
	100.0	100.0	36.2	55.3	100.0	96.0		50.0
	100.0	94.7	68.3	58.8	79.2	93.8		0.0
	0.0	84.5	42.2	60.6	100.0	100.0		
		32.6	66.0	66.7	97.1	56.7		
		15.8	48.5	43.5		89.7		
		54.5	35.3	95.5		64.0		
		100.0	38.9					
		19.0	78.3					
		29.4	85.7					
		96.0	70.6					
		80.8	100.0					
		90.0	19.4					
		75.0	42.2					
			61.9					
Mean %	51.7%	65.7%	55.3%	69.0%	98.3%	85.8%	55.1%	23.7%

An arcsine transformation of the data above was made, the means above were computed, and an Anova carried out:

Source of Variation	df	MS	F	
Among individuals	7	1913.2415	3.9134	P<0.010
C7, 8, ... 16 vs Others	1	4214.2313	8.6199	P<0.010
C9 vs C11	1	912.0849	1.8656	ns
C7 vs C8 vs ... vs C16	5	1653.2749	3.3817	P<0.010
Within individuals	72	488.8929		
Total	79			

APPENDIX C-4

31° INCUBATION: PERCENT HATCH

Female's hatching temperature	21°	27°	31°					
Individual	D8	D5	D3	D11	D16	D17	D19	D20
	0.0% 25.0 84.6 61.1 21.4	0.0% 41.2 65.4 42.3	-----	17.2% 45.0 41.4 82.9 100.0	44.4% 100.0 0.0 53.8 88.0 69.2 90.3 52.6	58.3%	60.0% 65.0 42.9 87.0	64.7% 66.7 23.1 100.0 25.0 61.5 90.0 0.0
Mean %	33.2%	30.7%	-----	62.8%	63.6%	58.3%	64.7%	54.4%

An arcsine transformation of the above data was made, the means above were computed, and an Anova carried out:

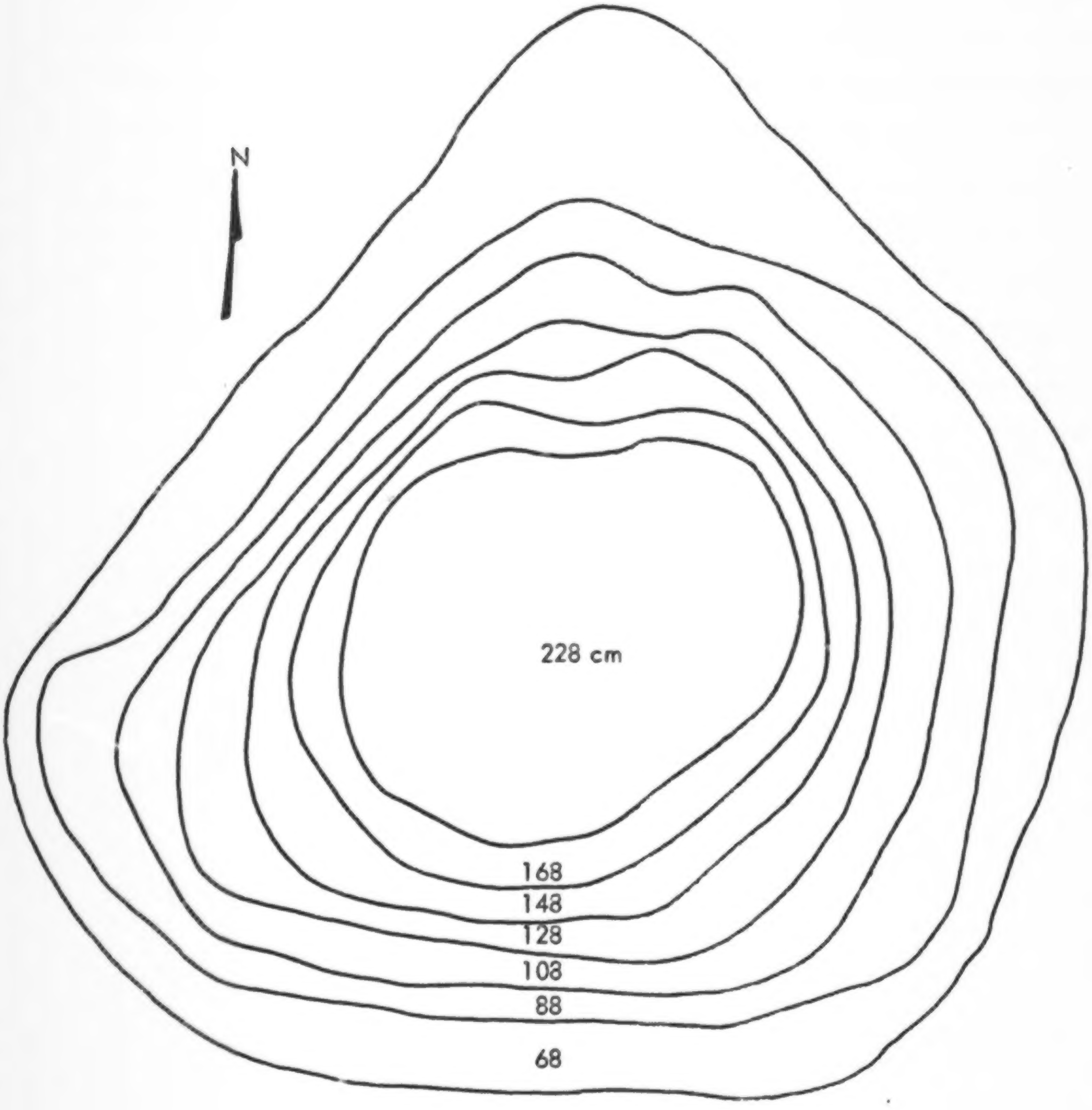
Source of Variation	df	MS	F	
Among individuals	6	337.7368	0.5347	ns
Within individuals	28	631.5237		
Total	34			

APPENDIX D

All measurements pertaining to the morphometry of the pond were determined following procedures outlined by Welch (1948). These include construction of the morphometric map (Appendix D-1), determination of the surface area and volume for each depth stratum (Appendix D-2), and the determination of surface area (Appendix D-3) and volume (Appendix D-4) of each region of the pond on each collecting date. The two regions of the pond are described in the Methods and Materials section of Chapter 4. On each collecting date the depth of the pond and the depth of the water at the junction of the open water region and the area of rooted aquatics was measured. By subtracting the depth of the water at the junction of the two regions from the total depth of the pond the appropriate dimensions of the open water area could be calculated.

APPENDIX D-1

MORPHOMETRIC MAP OF THE POND SHOWING DEPTH CONTOURS IN CENTIMETERS. THE SCALE IS 1:200.


N
228 cm
168
148
128
108
88
68

APPENDIX D-2

SURFACE AREA AND VOLUME OF THE VARIOUS DEPTH STRATA OF THE POND

Depth stratum (cm)	Surface area (cm^2)	Volume (1)
0- 68	9,536,250	981,996
68- 88	7,247,250	413,116
88-108	5,689,500	284,058
108-128	3,898,000	188,746
128-148	2,827,750	121,774
148-168	2,162,000	72,025
168-228	1,683,500	33,670

APPENDIX D-3

NUMBER OF QUADRATS IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE SURFACE AREA CAN BE COMPUTED BY MULTIPLYING THE NUMBER OF QUADRATS BY 45.54 CM^2

Date	Open water region	Rooted aquatic region	Total Pond
II-1971			
19	86,965	86,965	173,931
21	92,508	92,508	185,016
23	91,769	91,769	183,538
25	94,355	94,355	188,711
27	94,355	94,355	188,711
III-1971			
1	93,247	93,247	186,494
3	93,247	93,247	186,494
5	90,660	90,660	181,321
7	90,660	90,660	181,321
9	89,921	89,921	179,843
11	89,921	89,921	179,843
13	89,921	89,921	179,843
15	89,182	89,182	179,364
17	89,182	89,182	179,364
19	89,182	89,182	179,364
21	88,813	88,813	177,626
23	87,867	87,867	175,734
25	86,962	86,962	173,925
27	86,585	86,585	173,171
29	86,208	86,208	172,417
31	85,857	85,857	171,714
IV-1971			
2	115,099	55,107	170,206
4	109,198	59,551	168,749
6	99,363	67,878	167,241
8	95,429	71,108	166,538
10	91,495	73,534	165,030
12	81,409	81,409	162,819
14	73,746	86,862	160,608

APPENDIX D-3 (Cont.)

NUMBER OF QUADRATS IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE SURFACE AREA CAN BE COMPUTED BY MULTIPLYING THE NUMBER OF QUADRATS BY 45.54 CM^2

Date	Open water region	Rooted aquatic region	Total pond
IV-1971			
16	70,092	90,516	160,608
18	55,973	104,634	160,608
20	54,088	109,494	163,573
24	55,728	105,633	161,362
V-1971			
4	45,234	119,042	164,277
18	48,936	111,671	160,608
28	47,474	113,133	160,608
VI-1971			
11	79,719	105,261	184,981
25	106,575	84,335	190,910
VII-1971			
9	39,594	141,718	181,312
23	30,806	141,211	172,417
VIII-1971			
6	18,483	150,265	168,749
20	16,019	146,700	162,819
IX-1971			
3	16,019	145,343	161,362
17	18,360	143,002	161,362
30	18,360	144,459	162,819
X-1971			
14	18,360	144,459	162,819
21	12,800	157,406	170,206
29	7,344	162,862	170,206

APPENDIX D-4

VOLUME IN LITERS OF EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE

Date	Open water region	Rooted aquatic region	Total pond
II-1971			
19	429,690	150,739	580,429
21	504,605	201,313	705,919
23	496,246	192,942	689,188
25	532,242	215,506	747,748
27	532,242	215,506	747,748
III-1971			
1	485,087	237,562	722,649
3	485,087	237,562	722,649
5	477,640	186,448	664,089
7	477,640	186,448	664,089
9	469,500	177,857	647,358
11	469,500	177,857	647,358
13	469,500	177,857	647,358
15	457,366	173,261	630,627
17	457,366	173,261	630,627
19	457,366	173,261	630,627
21	451,349	170,910	622,259
23	438,341	167,187	605,528
25	429,676	150,753	580,429
27	423,790	148,277	572,067
29	417,938	145,760	563,699
31	412,247	143,089	555,336
IV-1971			
2	469,895	68,705	538,600
4	444,849	77,020	521,869
6	425,731	79,407	505,138
8	408,138	88,632	496,770
10	390,545	89,494	480,039
12	358,480	96,459	454,940
14	332,976	105,233	438,209
16	320,128	109,718	429,847
18	284,478	145,369	499,847

APPENDIX D-4 (Cont.)

VOLUME IN LITERS OF EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE

Date	Open water region	Rooted aquatic region	Total pond
IV-1971			
20	281,736	181,572	463,308
24	285,380	152,829	438,209
V-1971			
4	230,704	240,972	471,677
18	257,514	172,332	429,847
28	247,147	182,699	429,847
VI-1971			
11	462,438	243,481	705,919
25	593,881	178,966	772,847
VII-1971			
9	266,846	397,243	664,089
23	207,631	356,067	563,699
VIII-1971			
6	137,205	384,664	521,869
20	115,993	338,947	454,940
IX-1971			
3	114,534	323,675	438,209
17	127,927	310,282	438,209
30	129,599	325,341	454,940
X-1971			
14	129,599	325,341	454,940
21	102,011	436,589	538,600
29	61,873	476,726	538,600

APPENDIX E-1

TOTAL NUMBER OF ADULTS (CVI) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\bar{Y}$ (MEAN) NUMBER OF ADULTS PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatics region	Total pond
II-1971			
19	1,052,454	263,113	1,315,568
21	300,651	346,905	647,556
23	1,295,112	127,198	1,422,556
25	1,086,969	312,214	1,399,183
27	778,387	247,668	1,026,056
III-1971			
1	648,656	342,018	990,675
3	1,130,623	256,430	1,387,053
5	1,002,468	554,306	1,556,775
7	813,768	754,800	1,568,568
9	1,002,860	146,122	1,168,982
11	802,295	281,005	1,090,301
13	1,067,820	438,368	1,506,189
15	1,047,897	379,026	1,426,924
17	1,696,500	70,687	1,767,187
19	1,189,906	294,531	1,484,437
21	1,121,267	199,829	1,321,096
23	735,888	801,789	1,537,678
25	1,521,848	76,092	1,597,940
27	1,114,793	454,575	1,569,369
29	366,308	1,009,878	1,376,264
31	1,083,947	139,517	1,223,465
IV-1971			
2	1,251,705	13,887	1,265,593
4	668,839	89,326	758,166
6	1,068,160	161,210	1,229,371
8	1,526,877	26,665	1,553,543
10	1,749,859	32,682	1,782,542

APPENDIX E-1 (Cont.)

TOTAL NUMBER OF ADULTS (CVI) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\overline{Y}$ (MEAN) NUMBER OF ADULTS PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatic region	Total pond
IV-1971			
12	1,641,783	54,006	1,695,789
14	1,908,190	21,715	1,929,906
16	1,690,980	101,830	1,792,811
18	643,697	104,634	748,332
20	1,886,343	41,056	1,927,399
24	1,497,714	26,408	1,524,122
V-1971			
4	2,572,700	178,565	2,751,264
18	911,433	781,697	1,693,130
28	344,186	14,141	358,327
VI-1971			
11	1,813,607	118,418	1,932,025
25	4,782,553	0	4,782,553
VII-1971			
9	1,994,547	106,288	2,100,835
23	4,181,914	388,330	4,570,244
VIII-1971			
6	1,827,506	957,939	2,785,445
20	1,521,805	1,173,600	2,695,405
IX-1971			
3	1,085,287	2,579,838	3,665,125
17	940,950	858,012	1,798,962
30	348,840	1,137,614	1,486,454
X-1971			
14	218,025	415,319	633,344
21	17,600	255,784	273,384
29	25,704	346,081	371,785

APPENDIX E-2

TOTAL NUMBER OF COPEPODITE FIVE (CV) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\bar{Y}$ (MEAN) NUMBER OF CV PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatics region	Total pond
II-1971			
19	0	0	0
21	0	0	0
23	0	0	0
25	0	0	0
27	0	0	0
III-1971			
1	0	0	0
3	0	0	0
5	0	0	0
7	0	0	0
9	0	0	0
11	0	0	0
13	0	0	0
15	0	0	0
17	0	0	0
21	0	0	0
23	0	0	0
25	0	0	0
27	0	0	0
29	21,552	49,262	70,814
31	233,041	71,547	304,588
IV-1971			
2	766,921	41,331	818,251
4	286,646	126,546	413,192
6	633,444	263,028	894,472
8	966,227	8,889	975,116
10	972,144	9,192	981,336

APPENDIX E-2 (Cont.)

TOTAL NUMBER OF COPEPODITE FIVE (CV) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\overline{Y}$ (MEAN) NUMBER OF CV PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatics region	Total pond
IV-1971			
12	1,037,973	30,529	1,068,502
14	719,028	0	719,028
16	718,448	33,944	752,391
18	279,868	13,079	292,948
20	635,542	0	635,542
V-1971			
4	327,948	89,282	417,230
18	183,512	125,630	309,143
28	89,015	0	89,015
VI-1971			
11	1,863,449	26,315	1,889,764
25	1,372,163	21,083	1,393,247
VII-1971			
9	148,478	0	148,478
23	396,630	40,345	436,976
VIII-1971			
6	221,805	93,916	315,721
20	104,125	55,012	159,137
IX-1971			
3	68,081	218,014	286,096
17	119,341	53,625	172,967
30	6,845	144,459	151,344
X-1971			
14	44,589	72,229	116,818
21	8,000	196,758	204,758
29	11,934	81,431	93,365

APPENDIX E-3

TOTAL NUMBER OF COPEPODITE FOUR (CIV) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\overline{Y}$ (MEAN) NUMBER OF CIV PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatics region	Total pond
II-1971			
19	0	0	0
21	0	0	0
23	0	0	0
25	0	0	0
27	0	0	0
III-1971			
1	0	0	0
3	0	0	0
5	0	0	0
7	0	0	0
9	0	0	0
11	0	0	0
13	0	0	0
15	0	0	0
17	0	0	0
19	0	0	0
21	0	0	0
23	0	21,967	21,967
25	239,148	0	239,148
27	507,146	284,496	791,642
29	290,955	2,142,909	2,433,864
31	723,654	85,857	809,511
IV-1971			
2			
4	327,596	141,434	469,029
6	621,023	398,784	1,019,808
8	858,869	8,889	867,757

APPENDIX E-3 (Cont.)

TOTAL NUMBER OF COPEPODITE FOUR (CIV) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\bar{Y}$ (MEAN) NUMBER OF CIV PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatics region	Total pond
IV-1971			
10	823,463	0	823,463
12	783,568	10,176	793,744
14	516,226	0	516,226
16	464,363	0	464,363
18	118,944	26,159	145,103
20	610,429	13,686	624,115
V-1971			
4	197,900	74,401	272,301
18	336,439	348,974	685,414
28	41,540	0	41,540
VI-1971			
11	2,072,713	78,946	2,151,659
25	785,996	0	785,996
VII-1971			
9	287,058	17,714	304,773
23	134,777	0	134,777
VIII-1971			
6	95,499	56,349	151,848
20	50,060	48,899	98,959
IX-1971			
3	40,048	124,579	164,627
17	58,140	115,285	173,426
30	0	41,273	41,273
X-1971			
14	34,425	126,402	160,827
21	5,485	118,054	123,540
29	4,590	61,073	65,663

APPENDIX E-4

TOTAL NUMBER OF COPEPODITE THREE (CIII) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\bar{Y}$ (MEAN) NUMBER OF CIII PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatics region	Total pond
II-1971			
19	0	0	0
21	0	0	0
23	0	0	0
25	0	0	0
27	0	0	0
III-1971			
1	0	0	0
3	0	0	0
5	0	0	0
7	0	0	0
9	0	0	0
11	0	0	0
13	0	0	0
15	0	0	0
17	44,591	11,148	55,739
19	66,887	11,148	78,035
21	388,558	25,375	413,933
23	285,572	329,502	615,074
25	793,535	32,611	826,146
27	655,578	556,623	1,212,201
29	420,269	1,490,183	1,910,452
31	1,018,021	157,405	1,175,426
IV-1971			
2			
4	368,545	37,219	405,764
6	285,671	93,333	379,003
8	846,940	8,889	855,828
10	651,909	0	651,909

APPENDIX E-4 (Cont.)

TOTAL NUMBER OF COPEPODITE THREE (CIII) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\bar{Y}$ (MEAN) NUMBER OF CIII PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatics region	Total pond
IV-1971			
12	651,909	0	651,909
14	221,240	0	221,240
16	245,324	0	245,324
18	174,918	39,238	214,156
20	517,706	0	517,706
V-1971			
4	175,282	59,521	234,804
18	220,214	348,974	569,189
28	47,474	28,283	75,758
VI-1971			
11	1,265,551	39,473	1,305,024
25	586,167	0	586,167
VII-1971			
9	296,957	0	296,957
23	84,717	20,172	104,889
VIII-1971			
6	33,886	0	33,886
20	40,048	146,700	186,748
IX-1971			
3	26,031	207,632	233,634
17	67,320	35,750	103,071
30	24,480	168,535	193,015
X-1971			
14	36,720	180,574	217,294
21	7,314	0	7,314
29	7,344	61,073	68,417

APPENDIX E-5

TOTAL NUMBER OF COPEPODITE TWO (II) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\overline{Y}$ (MEAN) NUMBER OF CII PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatics region	Total pond
II-1971			
19	0	0	0
21	0	0	0
23	0	0	0
25	0	0	0
27	0	0	0
III-1971			
1	0	0	0
3	0	0	0
5	0	0	0
7	0	0	0
9	0	0	0
11	0	0	0
13	0	0	0
15	38,221	0	38,221
17	568,540	11,148	579,688
19	925,271	78,035	1,003,306
21	810,421	253,752	1,064,173
23	263,602	219,668	483,270
25	293,499	43,481	336,981
27	606,101	272,126	878,228
29	495,702	886,721	1,382,422
31	331,163	85,857	417,021
IV-1971			
2	1,107,831	158,434	1,266,265
4	709,790	44,663	754,454
6	335,353	25,454	360,807
8	477,149	8,889	486,038
10	194,429	9,192	203,621

APPENDIX E-5 (Cont.)

TOTAL NUMBER OF COPEPODITE TWO (II) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\overline{Y}$ (MEAN) NUMBER OF CII PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatics region	Total pond
IV-1971			
12	325,639	10,176	335,815
14	73,747	0	73,747
16	330,434	12,930	343,366
18	188,911	254,113	443,024
20	664,518	13,686	678,204
V-1971			
4	378,837	0	378,837
18	250,800	265,220	516,021
28	23,737	0	23,737
VI-1971			
11	1,145,971	78,946	1,224,917
25	306,405	10,541	316,947
VII-1971			
9	113,833	0	113,833
23	83,616	0	83,616
VIII-1971			
6	43,128	0	43,128
20	64,077	122,249	186,326
IX-1971			
3	12,114	62,289	74,304
17	39,780	71,501	111,281
30	104,041	385,224	489,265
X-1971			
14	32,130	18,057	50,187
21	3,657	59,027	62,684
29	1,836	0	1,836

APPENDIX E-6

TOTAL NUMBER OF COPEPODITE ONE (I) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\bar{Y}$ (MEAN) NUMBER OF CI PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatics region	Total pond
II-1971			
19	0	0	0
21	0	0	0
23	0	0	0
25	0	0	0
27	0	0	0
III-1971			
1	0	0	0
3	0	0	0
5	0	0	0
7	0	0	0
9	0	0	0
11	0	0	0
13	67,441	11,240	78,682
15	535,097	55,739	590,836
17	624,279	78,035	702,314
19	590,836	122,626	713,462
21	588,388	177,626	766,014
23	637,038	208,685	845,723
25	271,759	10,870	282,629
27	284,496	86,586	371,082
29	775,881	812,827	1,588,708
31	760,449	57,238	817,687
IV-1971			
2	776,921	110,215	887,136
4	696,141	7,444	703,584
6	273,250	25,454	298,705
8	262,432	17,777	280,209
10	194,429	0	194,429
12	437,577	10,176	447,753

APPENDIX E-6 (Cont.)

TOTAL NUMBER OF COPEPODITE ONE (I) IN EACH REGION OF THE POND AND IN THE TOTAL POND ON EACH COLLECTING DATE. THE TOTAL NUMBER WAS DETERMINED BY MULTIPLYING THE $\bar{Y}$ (MEAN) NUMBER OF CI PER QUADRAT IN EACH REGION BY THE TOTAL NUMBER OF QUADRATS IN THAT REGION AND SUMMING THE PRODUCTS FOR EACH DATE.

Date	Open water region	Rooted aquatics region	Total pond
IV-1971			
14	92,183	10,858	103,041
16	480,634	38,793	519,426
18	118,944	134,530	253,474
20	494,525	0	494,525
V-1971			
4	390,145	0	390,145
18	220,214	181,466	401,681
28	41,540	0	41,540
VI-1971			
11	966,601	26,315	992,917
25	213,151	0	213,151
VII-1971			
9	79,188	0	79,188
23	88,017	0	88,017
VIII-1971			
6	80,096	18,783	98,879
20	74,089	48,899	122,988
IX-1971			
3	30,036	0	30,036
17	30,600	17,875	48,475
30	146,882	24,075	170,957
X-1971			
14	16,065	0	16,065
21	3,657	19,675	23,332
29	0	0	0

APPENDIX E-7

TOTAL NUMBERS OF NAUPLIAR SIX (NVI), NAUPLIAR FIVE (NV), NAUPLIAR FOUR (NIV), AND NAUPLIAR ONE THROUGH THREE (NI-NIII) ON EACH COLLECTING DATE.

Date	NVI	NV	NIV	NI-NII
II-1971				
19	0	86,965	347,862	1,739,310
21	0	1,017,589	740,065	1,942,671
23	367,076	367,076	367,076	458,845
25	0	94,355	471,778	1,604,046
27	0	377,422	1,037,912	3,868,582
III-1971				
1	0	466,236	932,473	2,237,935
3	93,247	93,247	279,741	2,331,182
5	0	0	45,330	158,656
7	0	0	45,330	1,613,761
9	44,960	112,402	629,452	1,528,670
11	449,609	359,687	1,079,061	4,586,009
13	1,528,669	809,295	1,618,592	5,754,992
15	989,139	899,217	2,967,418	6,834,053
17	1,978,278	1,348,826	1,618,591	4,316,244
19	1,162,982	629,452	989,139	3,417,026
21	1,592,451	921,945	2,346,770	3,687,782
23	615,071	527,203	1,581,611	3,778,294
25	869,627	347,851	1,304,441	2,608,883
27	346,343	865,858	1,125,616	2,510,990
29	862,089	1,206,925	1,120,716	2,327,642
31	772,713	858,570	772,713	2,232,282
IV-1971				
2	680,827	1,191,447	1,872,275	2,638,206
4	506,248	590,623	928,122	2,278,118
6	83,620	250,862	668,967	2,675,871
8	416,346	333,076	416,346	1,332,307
10	330,061	330,061	247,546	1,072,700
12	407,049	569,869	488,459	2,605,115
14	240,912	562,130	401,521	1,124,260
16	0	160,608	562,130	562,130
18	401,521	240,912	240,912	481,825
20	490,720	245,360	81,786	817,867

APPENDIX E-7 (Cont.)

TOTAL NUMBERS OF NAUPLIAR SIX (NVI), NAUPLIAR FIVE (NV), NAUPLIAR FOUR (NIV), AND NAUPLIAR ONE THROUGH THREE (NI-NIII) ON EACH COLLECTING DATE.

Date	NVI	NV	NIV	NI-NIII
V-1971				
4	523,302	320,205	465,211	887,493
18	321,217	160,608	321,217	883,347
28	0	0	80,304	160,608
VI-1971				
11	647,433	1,109,886	1,387,358	1,757,320
25	190,910	0	95,455	286,366
VII-1971				
9	0	362,625	271,968	815,906
23	517,253	258,626	603,462	2,069,015
VIII-1971				
6	421,873	337,499	590,623	1,181,246
20	569,869	651,278	895,508	3,419,214
IX-1971				
3	242,043	161,362	80,695	806,812
17	0	0	0	0
X-1971				
14	0	81,409	162,819	407,049
21	0	0	0	170,206
29	0	0	85,103	0

APPENDIX E-8

TOTAL NUMBER OF EGGS IN THE POND ON EACH COLLECTING DATE. THE TOTAL NUMBER OF EGGS WAS DETERMINED BY MULTIPLYING THE $\bar{Y}$ (MEAN) NUMBER OF EGGS PER ADULT BY THE TOTAL NUMBER OF ADULTS IN THE POPULATION ON EACH DATE.

Date	Number of eggs (X 10^3)
II-1971	
19	7,631
21	1,359
23	6,561
25	6,955
27	9,892
III-1971	
1	10,385
3	17,134
5	15,646
7	10,454
9	11,060
11	8,295
13	16,186
15	18,687
17	15,644
19	19,048
21	20,557
23	16,277
25	23,890
27	35,243
29	18,793
31	18,602
IV-1971	
2	19,444
4	10,565
6	12,650
8	16,674
10	11,849
12	4,753
14	2,570
16	2,229
18	3,416
IV-1971	
20	5,905
24	6,028
V-1971	
4	11,220
18	4,505
28	1,511
VI-1971	
11	7,162
25	27,339
VII-1971	
9	11,845
23	29,489
VIII-1971	
6	17,844
20	9,560
IX-1971	
3	21,832
17	11,689
30	5,310
X-1971	
14	433
21	0
29	744

APPENDIX F-1

PERCENTAGE OF CYCLOPS VERNALIS NAUPLII SURVIVING 24 AND 48 HOURS AFTER HATCHING WITH AND WITHOUT THE PRESENCE OF THE MOTHER.

Clutch No.	Female present Number of nauplii surviving					Clutch No.	Female removed at time 0 Number of nauplii surviving				
	0hr	24 hr.	%	48 hr.	%		0hr	24 hr.	%	48 hr.	%
1	83	25	30	10	12	1	76	73	97	73	97
2	68	33	49	26	38	2	95	91	96	84	88
3	49	30	61	29	59	3	59	59	100	58	98
4	55	33	60	20	36	4	52	52	100	51	98
5	32	19	59	15	47	5	24	24	100	22	92
6	65	54	83	41	63	6	38	37	97	36	95
7	51	24	47	18	35	7	96	96	100	96	100
8	73	34	47	28	38	8	26	26	100	26	100
9	46	16	35	13	28	9	57	55	96	55	96
10	50	34	68	31	62	10	50	49	98	49	98
Mean			53.9		41.8				98.4		96.2
$SE_{\bar{x}}$			±4.95		±5.13				±0.56		±1.18
No.			10		10				10		10

APPENDIX G-1

DURATIONS IN DAYS OF THE ADULT STAGE, THE PERIODS FROM MATURATION TO THE PRODUCTION OF THE FIRST EGG CLUTCH, THE PERIODS FROM THE PRODUCTION OF THE LAST EGG CLUTCH TO DEATH AND THE PERIODS BETWEEN THE PRODUCTION OF SUCCESSIVE EGG CLUTCHES FOR *CYCLOPS VERNALIS* FEMALES AT TEMPERATURES OF 14, 21, 26, and 31° C.

	Total adult	Before eggs	After eggs	Between clutches	Total adult	Before eggs	After eggs	Between clutches
	14				21			
	77	7	12	11.6	52	15	12	6.3
	74	33	5	9.5	37	11	4	7.7
	69	38	20	10.0	51	7	10	6.8
	58	11	9	7.6	43	6	37	---
	84	18	7	11.0	63	30	9	6.0
	83	57	10	14.0	64	37	13	14.0
					59	6	10	6.1
					55	31	3	10.5
					76	27	6	9.0
					57	26	10	6.4
					59	31	3	6.0
					67	33	8	5.3
					60	51	4	8.0
Mean	38.5	12.9	6.3	3.65	26.2	8.3	6.9	3.62
$SE_{\bar{x}}$	2.49	1.97	1.31	0.268	0.72	1.06	0.88	0.335
n	11	11	11	11	19	19	19	17
	26				31			
	39	26	1	4.0	26	14	4	4.0
	39	22	7	3.0	25	2	1	4.4
	32	10	2	4.8	26	5	5	3.2
	37	3	3	2.6	30	2	6	5.5
	59	16	6	3.5	27	10	5	4.0
	40	9	1	3.6	22	8	11	4.5
	37	13	12	3.0	24	7	7	5.0
	30	7	11	2.4	29	1	4	6.7
	28	12	11	5.0	25	9	6	3.3
	38	13	11	4.7	25	16	7	2.0
	44	11	4	3.6	27	13	8	3.0
					20	8	11	---
					28	13	8	1.8
					22	16	4	2.0
					23	10	12	---
					29	7	5	2.8
					32	8	6	4.5
					28	4	18	2.0
					30	4	4	2.8
Mean	74.2	27.3	10.5	10.62	57.2	23.9	9.9	7.68
$SE_{\bar{x}}$	±3.96	±7.73	±2.14	±0.881	±2.80	±3.85	±2.45	±0.717
n	6	6	6	6	13	13	13	13

APPENDIX H-1

FECUNDITY IN EGGS PER CLUTCH OF FEMALE *CYCLOPS VERNALIS* AT 14° C.

Clutch No.	Female No.					
	1	2	3	4	5	6
1	21	61	25	67	39	24
2	32	75	22*	56	61	12
3	49	63		51	53	
4	28	54		51	29	
5	22	24		44	28	
6	11			41	24	
Total eggs per female	163	277	47	310	234	36
No. of clutches per female	6	5	2	6	6	2

*Only one of two possible egg sacs present.

APPENDIX H-2

FECUNDITY IN EGGS PER CLUTCH OF FEMALE CYCLOPS VERNALIS AT 21° C.

Clutch No.	Female No. 1	2	3	4	5	6	7	8	9	10	11	12	13
1	101	58	69	3*	40	55	60	81	69	42	43	73	71
2	60	54	55		50	74	62	102	81	10	29	43	74
3	81	57	51		36		63	51	68	13	36	47	50
4	71	6*	50		28		52		62	13*	44	56	
5	37		48		33		64		12	36	29	46	
6			23				43		27*	27	48	29	
7							29		12*		34	28	
8							15					26	
Total eggs per female	350	175	296	3	187	129	388	234	331	141	263	348	195
No. of clutches per female	5	4	6	1	5	2	8	3	7	6	7	8	3

*Only one of two possible egg sacs present.

APPENDIX H-3

FECUNDITY IN EGGS PER CLUTCH OF FEMALE *CYCLOPS VERNALIS* AT 26° C.

Clutch No.	Female No.										
	1	2	3	4	5	6	7	8	9	10	11
1	37	42	49	56	48	22*	34	33	6*	54	14
2	35	31	70	62	51	30	31	28	19	47	13*
3	(28)	17	66	63	45	10	25	18		35	20*
4	13	19	61	66	(37)	18	17	32		15	17*
5			23	48	44	21	26	21			16*
6				(48)	30	16		26			13*
7				35	32	5*					10*
8				38	28	5*					15
9				14*	23*	13					6*
Total eggs per female	113	109	269	430	338	140	133	158	25	151	124
No. of clutches	4	4	5	9	9	9	5	6	2	4	9

*Only one of two possible egg sacs present.

() Egg count not available, number is average clutch size for that female.

APPENDIX H-4

FECUNDITY IN EGGS PER CLUTCH OF FEMALE CYCLOPS VERNALIS AT 31°C

Clutch No.	Female No.																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	19	31	44	48	50	47	18*	27	39	9*	33	36	29	29	4*	18	33	62	59
2	4*	37	42	46	47	31	27	31	31	20	37		33	25		36	29	53	39
3	19	52	28	15	20	28	20	17	24		27		27			45	36	38	32
4		31	32	22	9			7*	23				18			47	25	38	51
5		33	(34)	14												46	20		50
6		24	24													35			51
7																15			46
8																			12
9																			16
Total eggs per female	42	208	204	145	126	106	65	82	117	29	97	36	107	54	4	242	143	191	356
No. of clutches per female	3	6	6	5	4	3	3	4	4	2	3	1	4	2	1	7	5	4	9

*Only one of two possible egg sacs present.

() egg count not available, number is average clutch size for that female.

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